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RELAPSE OF PATIENTS WITH PERNICIOUS ANEMIA RECEIVING FOLIC ACID

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The recent medical literature contains many articles which report the efficacy of folic acid in the treatment of macrocytic anemias,[2,4,5] the anemias of childhood with a megaloblastic bone marrow.[10] The refractory anemias, and leukopenic states,[9] in the relief of pellagrous glossitis,[6] and particularly in the treatment of pernicious anemia.[1]

When a well-recommended remedy enters the therapeutic field, a tendency soon develops to look upon it as a non-failing curative agent. This is particularly true if it is described as a specific drug for a specific disease. Many practitioners and a host of misinformed laymen believe today that folic acid is, at least, an unfailing medicine for the treatment of pernicious anemia.[8] This is a misconception which can lead to disastrous consequences. For this reason we report 2 patients with pernicious anemia who relapsed while receiving folic acid.

Case Reports. Case 1. (Duke Unit A 57768.) A 38 year old salesman, first seen in March 1941, complaining of anemia, recognized 2 years previously. The physical examination showed a prematurely graying, well developed, well nourished, non-icteric man, with a moderate deflection of the nasal septum, with a right, indirect inguinal hernia, and a mild dermatophytosis of both feet. There was no papillary atrophy of the tongue. The neurologic examination was entirely normal. The gastric analysis disclosed a complete lack of free HCl after the administration of histamine, a total acidity of 7°. The blood serology, the urine, the blood chemical studies, the basal metabolism and the Roentgen examinations of the chest and gastro-intestinal tract showed nothing abnormal.

Blood Picture. Hgb., 11.2 gm. (72%); RBC, 2,510,000; WBC, 5450; Col.In., 1.44; MCV, 130 cμ; retics., 4%; platelets normal. The patient received 1 cc. of a potent concentrated liver extract intramuscularly each day for 10 days. On the 5th day the reticulocytes were 18% of the total red cell count; the total blood values and indices were essentially the same as on admission. He was subjectively improved so that he no longer complained of weakness and fatigue. He was discharged and was advised to take 15 units of liver extract intramuscularly twice a week for 6 weeks, and oral liver extract 1 tablespoonful 3 times daily until his blood levels were normal. Since the patient's brother was a practising physician it was hoped that this plan of treatment would be carried out faithfully. The patient was readmitted to this hospital on May 27, 1946 because of a return of weakness, fatigability, exertional dyspnea and, in addition, soreness of the tongue of 6 months duration. He stated at this time that he had discontinued the use of parenteral liver extract

3 years previously, and had taken oral liver preparations only sporadically. The general examination showed that he was still well nourished; the mucous membranes were pale, the skin had a lemon yellow tint. There was still no frank jaundice, there was no evidence of bleeding, there was no atrophy of the papillæ of the tongue, the heart and lungs were normal, the liver and spleen were not palpable; B.P. 100/72. The neurologic examination showed nothing abnormal.

This patient responded fairly well to 30 mg. of folic acid a day; he maintained his hemoglobin and red cell levels for 5 months on 5 to 10 mg. of folic acid a day, and then relapsed. The relapse proceeded despite increased doses of folic acid. This is shown in the following table.

TABLE 1.—BLOOD CELL DATA IN CASE 1

Date	Hgb. (gm.)	RBC (mills.)	WBC (thous.)	Hem. (vol. %)	C.I	MCHC ($\times 10^{-12}$ gm.)	MCV ($c\mu$)	Retics. (%)	Treatment
1946									
5/27	9.8	2.64	4.35	29.0	1.11	37.0	110.0	4.3	5/29, 10 mg. folic acid t.i.d.
5/30	..	..	..	..	..	..	..	8.0	
5/31	..	..	..	..	..	..	..	5.0	
6/1	..	..	..	..	..	..	..	3.1	
6/3	..	..	..	..	..	..	..	2.6	
6/4	11.0	2.97	4.00	30.2	1.22	36.6	106.6	3.0	6/5, 10 mg. folic acid b.d.
6/21	14.1	4.11	10.00	43.0	1.08	34.0	104.0	3.0	6/28, 5 mg. folic acid q.d.
7/20	16.7	4.52	5.20	46.0	1.20	36.0	102.0	1.0	
9/27	15.7	4.50	5.60	41.0	1.10	35.0	104.0	1.5	10 mg. folic acid q.d.
10/31	15.7	4.55	6.80	46.0	1.10	34.0	101.0	2.0	5 mg. folic acid q.d.
1947									
1/20	13.2	4.07	2.95	38.0	1.04	32.4	95.0	2.0	15 mg. folic acid t.i.d.
1/29	13.4	3.57	3.80	38.0	1.22	32.5	106.0	1.5	
2/3	12.1	2.90	3.60	32.0	1.32	36.0	110.0	1.0	Liver extract, 15 u. a day
2/9	13.2	3.30	6.80	..	1.14	35.0	105.0	12.8	Liver extract, 15 u. twice a week
2/21	14.8	4.25	5.95	41.0	1.10	37.0	92.0	1.5	

The bone marrow pattern was always consistent with the severity of the anemia, so that megaloblasts were present in abundance when the anemia was severe, and were sparse as the maturation of erythropoietic elements took place, and as the anemia subsided. This patient was discharged from the hospital and was instructed to take liver extract parenterally, 15 units twice a week for 3 weeks, and thereafter once a week or once every 2 weeks as necessary to maintain normal blood levels. When he was last heard from the blood levels were apparently normal, as were the indices, and a blood smear recently obtained failed to show macrocytosis.

CASE 2. (Duke Unit B 81542.) A 63 year old unemployed male who was admitted to this hospital on June 14, 1946, complaining of progressive weakness of 3 weeks duration. He was also short of breath on exertion. His appetite was good, and he denied gastro-intestinal disturbances. His tongue had been sore, off and on, for about 2 years, and he had noticed some numbness and tingling in the fingers and toes for about 3 months. He was pale and thin, the scleræ were bluish. The marginal papillæ of the tongue were atrophied. The heart and lungs were normal. B.P. 110/68. The liver was not palpable, but the tip of the spleen could be felt on deep inspiration. The vibratory sense (fork No. 250) was lost below the knee on the right side, and below the ankle on the left side. The blood serology, the urine and stool were normal. The blood NPN, uric acid and Van den Bergh were not elevated. The serum proteins were normal. Roentgen rays of the chest and of the gastro-intestinal tract were essentially normal, as was the electrocardiogram. The gastric analysis disclosed a complete lack of free HCl.

Blood Picture (June 15, 1946). Hgb., 4.4 gm. (28%); RBC, 1,210,000; WBC, 4000; retics., 7.1%. Anisocytosis and poikilocytosis were striking and many macrocytes were seen; there were a few erythroblasts (about 1 per 100 white cells). The differential white cell formula was normal. *Bone marrow:* The material obtained by sternal puncture was very cellular and the nucleated cell count was estimated to be 182,000 per mm.^{2} The erythroid elements were present in tremendous numbers and were mostly erythroblasts and megaloblasts. The reticulocyte count was 8%.

This patient had never received antiperni-

cious anemia therapy. He responded well to 30 mg. of folic acid a day, so that the reticulocyte count reached 29% on the 9th day. The hemoglobin and red cell levels never approached the adequate normal with doses of 20 and 10 mg. of folic acid a day. The hematologic relapse occurred after 90 days, even though the dosage of folic acid was increased from 5 to 30 mg. a day as shown in the second table.

treatment for his pernicious anemia, and, as far as could be determined, had not been treated with liver extract. He responded well to the oral administration of folic acid. The subjective improvement was also satisfactory. However, macrocytosis again persisted and the color index, mean corpuscular volume and the mean hemoglobin content did not revert to

TABLE 2.—BLOOD CELL DATA IN CASE 2.

Date	Hgb. (gm.)	RBC (mills.)	WBC (thous.)	Hem. (vol. %)	C.I.	MCHC ($\times 10^{-12}$ gm.)	MCV (cμ)	Retics. (%)	Treatment
1946									
6/15	4.4	1.21	4.0	13.0	1.20	36.4	109.0	7.1	
									6/16, folic acid, 10 mg. t.i.d.
6/20	5.0	1.50	11.9	..	1.06	33.3	113.0	9.6	
6/24	6.1	1.94	4.0	20.0	1.02	30.9	103.0	29.1	
6/27	7.5	2.35	4.4	25.5	1.01	31.0	108.0	9.0	
6/29	7.6	2.66	4.4	26.0	0.94	28.5	97.7	10.0	Folic acid, 10 mg. b.d.
									7/6, folic acid, 5 mg. b.d.
									7/20, folic acid, 5 mg. q.d.
7/29	10.7	3.60	6.5	38.5	0.96	30.0	107.0	1.5	
9/30	10.0	3.56	6.7	35.5	0.99	30.0	99.0	1.5	Folic acid, 5 mg. b.d.
10/30	8.9	2.85	4.9	29.5	1.01	31.0	103.0	1.5	Folic acid, 10 mg. t.i.d.
11/9	8.4	2.59	7.1	30.0	1.04	32.0	102.0	1.0	Liver extract, 15 u. q.d.
11/14	10.8	3.46	7.9	35.8	1.00	30.0	103.0	7.5	
11/22	11.5	3.87	11.0	39.5	0.94	29.7	101.0	5.5	Liver extract, 15 u. twice a week
1947									
1/29	14.5	4.35	7.2	41.5	1.07	28.0	90.0	1.5	

Discussion. These 2 patients were undoubtedly suffering from pernicious anemia, and the first patient had been proven previously to be responsive, clinically and hematologically, to liver fractions effective in pernicious anemia. When he relapsed, because he discontinued his anti-anemic liver extract therapy, he received folic acid and had a very satisfactory initial response. However, it is evident that the hematologic reaction never returned to normal. Although the reticulocyte reaction was adequate, and the red cell count and hemoglobin levels rose appreciably, the hematologic indices never reverted to normal, and the macrocytosis persisted. This is interesting in view of the reports of the efficacy of folic acid in patients with macrocytic anemia in relapse.[3,7] Furthermore, this patient continued to relapse after receiving large and increasing doses of folic acid, and then responded beautifully to 15 units of concentrated liver extract a day for 8 days.

The second patient had never received normal. Within a relatively short time, this patient relapsed while he was receiving 5 mg. and then 30 mg. of folic acid a day. He also reacted properly to standard amounts of concentrated liver extract given parenterally.

It is apparent that in some patients the accepted dosage of folic acid will not maintain a hematologic remission. In these 2 patients the initial response to folic acid was adequate, except for the persistence of macrocytosis. The continued macrocytosis does not necessarily imply a relapse. Thirty other patients with pernicious anemia were treated with folic acid in this clinic. In these patients, the initial reticulocyte response was adequate in 17, fair in 10 and unsatisfactory in 3. Macrocytosis, as proven by the appearance of the red cells in fresh and stained smears, and by the mean corpuscular volume, persisted in a high percentage of this group. This is being reported separately.

Folic acid, administered by mouth, therefore, seems to lack some of the prop-

erties of purified liver extract administered parenterally. In 2 patients with pernicious anemia studied in this clinic, folic acid failed to maintain a remission.

Summary and Conclusions. 1. Two patients are reported, who were suffering from pernicious anemia, and relapsed while receiving folic acid.

2. The hematologic response after folic acid therapy in these 2 patients was not complete insofar that macrocytosis persisted and the hematologic indices remained abnormal.

3. Both patients relapsed while receiving what is regarded as adequate folic acid therapy.

4. These 2 patients showed a subsequent satisfactory response to liver extract administered parenterally in doses considered minimal for the treatment of patients with pernicious anemia.

5. Folic acid in doses generally accepted as effective will not prevent a hematologic relapse in some patients with pernicious anemia.

REFERENCES

1. Berry, L. J., and Spies, T. D.: The Present Status of Folic Acid, Blood, **1**, 271, 1946.

2. Darby, W. J., Jones, E., and Johnson, H. C.: Effect of Synthetic *Lactobacillus casei* Factor in Treatment of Sprue, J. Am. Med. Assn., **130**, 780, 1946.

3. Frommeyer, W. B., Jr., and Spies, T. D.: Relative Clinical and Hematologic Effects of Concentrated Liver Extract, Synthetic Folic Acid and Synthetic 5-Methyl Uracil in the Treatment of Macrocytic Anemias in Relapse, Am. J. Med. Sci., **213**, 135, 1947.

4. Spies, T. D.: The Activity of another *L. casei* Factor in Macrocytic Anemia, South. Med. J., **39**, 634, 1946.

5. Spies, T. D., Lopez, G. G., Menendez, J. A., Minnich, V., and Koch, M. B.: The Effect of Folic Acid on Sprue, South. Med. J., **39**, 30, 1946.

6. Spies, T. D.: Relief of Pellagrous Glossitis With Synthetic Folic Acid, Am. J. Med., **1**, 473, 1946.

7. Spies, T. D.: Effect of Folic Acid on Persons With Macrocytic Anemia in Relapse, J. Am. Med. Assn., **130**, 474, 1946.

8. Totter, J. R.: Folic Acid Treatment of Pernicious Anemia is Effective, quoted by Durham Morning Herald, Durham, N. C., Dec. 11, 1946.

9. Watson, C. J., Sebrell, W. H., McKelbey, J. L., and Daft, F. S.: Possible Effectiveness of the *L. casei* Factor ("Folic Acid") Concentrates on Refractory Anemia and Leukopenia, With Particular Reference to Leukopenia Following Radiation Therapy, Am. J. Med. Sci., **210**, 463, 1945.

10. Zuelzer, W. W.: Folic Acid Therapy in the Anemias of Infancy and Childhood, J. Am. Med. Assn., **131**, 7, 1946.

THE DIAGNOSTIC VALUE OF THE STERNAL BONE MARROW PUNCTURE IN POLYCYTHEMIA VERA

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The diagnosis of polycythemia vera (erythremia) is not difficult when the clinical manifestations of the disease are well advanced, and abnormally high values for number of erythrocytes, volume of packed red cells and quantity of hemoglobin are found in the peripheral blood. In the early stages of the disease, however, there is no distinct borderline between the upper limits of normal values for erythrocytes in healthy individuals and the range of abnormal values for early polycythemia vera. Until a more exact definition of diagnostic criteria for the recognition of the early phases of the disease can be established, the diagnosis of these early cases must often remain speculative until the course of the disease reveals its true nature. An evaluation of the sternal marrow puncture as an aid in the diagnosis of polycythemia vera therefore seemed desirable.

Material. The clinical records and sternal bone marrow material of the Hematology Laboratory, Duke Hospital, was studied from all patients with the diagnosis of polycythemia vera. The cases were considered in 3 groups: (1) polycythemia vera; (2) suspected polycythemia vera; and (3) normal controls.

In Group 1 there are 22 patients in whom the diagnosis of polycythemia vera seemed clearly established on the basis of clinical, hematologic and other laboratory data. Some of this information is tabulated in Table 1. These patients, in general, had values ranging consistently above 6,000,000 per c.mm. for erythrocytes, above 18 gm. per 100 cc. for hemoglobin, and above 50 cc. per 100 cc. for volume of packed red cells. The clinical findings and course of the disease while under observation were thought typical of either essential polycythemia with splenomegaly or polycythemia with hypertension (Gaisböck's syndrome).

Group 2 includes 29 patients in whom a diagnosis of polycythemia vera was made or seriously considered, so much so that several patients in this group were treated by venesection or by irradiation using "spray" technique. Some of the clinical and laboratory data on this group are tabulated in Table 2. In most instances, however, this group of patients failed to maintain values for hemoglobin, number of erythrocytes and volume of packed red cells which were consistently above the upper ranges of normal values during the periods under observation. In addition, splenomegaly, hepatomegaly, plethoric appearance and other clinical features of polycythemia vera were often lacking. These patients appear to represent a mixed, borderline group including individuals with early polycythemia vera, normal individuals with values for erythrocytes, hemoglobin and volume of packed cells at the upper limits of normal range, and individuals with mild erythrocytosis of undetermined cause. Patients with demonstrable causes for erythrocytosis, such as heart disease, pulmonary disease and anoxemia from other conditions, were excluded from the study.

Group 3 represents a control group of 50 normal medical students and technicians ranging in age from 19 to 35 years. The peripheral blood data on this group was within normal range. Since the studies of several investigators[5] vary widely for normal values for sternal marrow, it was felt advisable to establish, for purposes of comparison, the normal values for sternal marrow by the techniques employed in this clinic.

Methods. The technique of sternal bone marrow puncture was uniform in all cases.

A No. 18 spinal puncture needle was introduced vertically into the body of the sternum between the levels of the second and third ribs under procaine anesthesia, and 1 cc. or less of sternal marrow was aspirated into a dry syringe. Smears were made immediately from oxalated marrow specimens in most of the earlier cases. Later, smears were made directly from fresh marrow from the sternal puncture needle at the bedside. The fixed smears were stained with a modification of Wright's stain. Counts of the total nucleated marrow cells were made and the total values of white blood cells calculated. Reticulated counts were made by the wet film technique. In almost all cases, the total counts and differential counts were made by the same experienced hematologic technician.

The limitations in the technique of sternal bone marrow puncture and the wide variations and different interpretations of the results are too well known to require elaboration. It is generally conceded that the sternal marrow puncture offers a safe clinical means by which valuable knowledge of bone marrow function may be secured.

TABLE 1.—SUMMARY OF DATA. INCLUDING PHYSICAL FINDINGS AND MAXIMAL OBSERVED VALUES IN THE PERIPHERAL BLOOD, IN 22 CASES OF POLYCYTHEMIA VERA

Case No.	Sex	Age	Plethoric appearance	Splenomegaly	Hepatomegaly	Blood pressure	R.B.C. (mill. per c.mm.)	Hb. (gm.)	Vol. packed cells (%)	W.B.C. (thous. per c.mm.)
1	M	57	+++	++++	++	110/90	9.95	25.6	58.0	21.35
2	F	38	++	++++	++++	130/100	8.0	21.0	64.0	17.8
3	F	72	+++	++	+++	140/86	8.28	22.5	65.0	20.8
4	M	52	+++	++++	++++	110/62	8.09	26.0	69.7	8.75
5	M	52	+++	++++	++++	118/88	7.66	16.1	53.4	34.0
6	M	39	++	+	+++	112/74	6.76	20.8	49.0	10.3
7	M	65	+++	++++	++	125/80	8.8	19.4	58.9	56.0
8	M	47	+++	0	+	150/82	6.83	21.2	56.7	11.3
9	M	47	+++	+++	+	132/86	8.75	23.0	80.8	9.0
10	F	53	+	0	+	230/126	7.0	22.0	63.2	10.44
11	F	42	+++	+	++++	236/148	6.64	20.3	64.0	16.0
12	M	48	++	++++	++++	190/128	6.64	20.0	56.0	8.2
13	M	30	+++	++++	+	140/90	8.76	25.5	67.6	15.65
14	M	58	++++	++++	++	148/86	8.0	21.1	57.7	20.0
15	M	24	++++	0	0	200/120	7.8	20.0	61.0	21.76
16	M	18	++	0	0	120/80	7.03	20.5	56.7	22.8
17	M	42	+++	0	+	150/100	6.68	24.8	75.2	9.8
18	M	47	+++	0	+	180/120	6.05	19.0	52.0	19.1
19	F	45	+++	++++	+++	160/80	9.05	25.0	83.9	17.4
20	F	51	+	++++	++++	110/70	7.04	17.0	51.23	11.0
21	M	68	+++	+	+++	148/94	7.79	19.0	60.9	21.8
22	M	35	+++	0	0	136/78	6.98	15.5	49.0	17.2

TABLE 2.—SUMMARY OF DATA, INCLUDING PHYSICAL FINDINGS AND MAXIMAL OBSERVED VALUES IN PERIPHERAL BLOOD, IN 29 CASES OF SUSPECTED POLYCYTHEMIA VERA

Case No.	Sex	Age	Plethoric appearance	Splenomegaly	Hepatomegaly	Blood pressure	R.B.C. (mill. per c.mm.)	Hb. (gm.)	Vol. packed cells (%)	W.B.C. (thous. per c.mm.)
1	M	47	++	0	0	120/80	6.29	20.2	50.4	9.20
2	M	41	++	0	0	140/90	6.49	18.2	53.0	6.49
3	M	36	+	0	0	110/80	6.0	18.6	57.0	11.30
4	M	33	+	++	0	130/78	5.85	17.5	53.0	13.05
5	M	37	++	0	0	120/70	6.64	18.0	51.23	11.55
6	M	64	+++	+	++++	160/102	5.77	17.3	..	6.95
7	M	28	0	0	+++	138/80	6.01	19.0	47.9	9.4
8	M	37	0	0	++	130/78	6.06	18.6	47.41	15.45
9	M	42	0	0		112/50	7.82	17.8	60.0	10.25
10	F	38	+++	0	0	150/90	5.85	19.0	4.4	15.7
11	M	55	+++	0	++++	150/85	6.04	18.8	50.0	9.5
12	M	65	0	0	0	112/70	6.12	19.0	54.5	11.2
13	M	48	++	++	+++	170/110	6.27	19.5	55.0	11.15
14	M	38	0	0	0	125/80	5.45	17.2	53.0	10.75
15	F	30	++	0	0	130/80	6.99	21.0	51.2	12.3
16	M	38	0	0	0	134/86	6.31	18.0	50.1	15.52
17	M	47	+	0	+++	140/80	5.56	17.3	..	8.75
18	M	43	+	0	++++	130/78	6.14	18.4	46.9	18.0
19	M	46	++	0	++++	138/96	5.97	19.2	51.0	13.15
20	M	39	0	0	0	130/86	5.28	16.1	48.0	16.5
21	M	26	+++	0	0	126/84	6.58	16.5	50.0	4.75
22	M	38	0	0	0	114/70	6.32	19.2	50.0	9.0
23	M	39	+	+	+	130/90	6.12	21.6	57.2	10.6
24	M	42	+	0	0	118/80	5.84	17.5	55.0	9.16
25	M	32	0	0	0	158/102	5.9	18.4	52.32	8.3
26	M	39	+	0	+++	120/80	6.09	19.0	49.0	9.1
27	M	69	+	0	++	210/130	5.72	18.0	53.0	11.9
28	M	38	+	0	++	180/124	6.23	19.1	58.8	9.1
29	M	19	0	0	0	180/120	5.95	19.0	54.5	10.45

RESULTS. The morphology of the sternal marrow of the 3 groups of cases was analyzed on the basis of comparative levels of total nucleated cells, total white cell count, differential counts on the white cell series, differential counts on the erythroid series, myeloid-erythroid ratios, and percentage levels of reticulocytes (see Tables 1 to 8).

Absolute counts of marrow cells are subject to wide fluctuations due to technical reasons, even in normal individuals, and are generally conceded to be of little value. To judge hyperplasia of the bone marrow cells, some reliance must be placed on the relative members of nucleated cells in the marrow specimen, whether this be based simply on visual impressions of number of nucleated cells in studying a microscopic field, or by actual count of the nucleated elements. In various hematologic disorders, the nucleated cells of the marrow are found to be increased or decreased beyond the average levels found in normal individuals. It is of considerable importance for clinical analysis to determine whether these variations from the normal involve the white cell series, the erythroid series, or both.

TABLE 3.—TOTAL NUCLEATED CELL COUNT IN STERNAL MARROW IN POLYCYTHEMIA VERA, SUSPECTED POLYCYTHEMIA, AND NORMAL INDIVIDUALS

Total nucleated count (thous. per c.mm.)	Group 1 Polycythemia vera (% at level indicated)	Group 2 Suspected polycythemia vera (% at level indicated)	Group 3 Normal controls (% at level indicated)
0–10	8.0	3.4	4.0
11–20	16.0	6.6	14.0
21–30	0	16.6	14.0
31–40	20.0	23.4	24.0
41–50	16.0	6.6	12.0
51–60	8.0	16.6	12.0
61–70	8.0	10.0	4.0
71–80	0	3.4	6.0
81–90	12.0	0	0
91–100	8.0	6.6	6.0
101–110	4.0	3.4	0
111–120	0	3.4	0
121–130	0	0	0
131–140	0	0	4.0

TABLE 4.—TOTAL WHITE CELL COUNT IN STERNAL MARROW IN POLYCYTHEMIA VERA, SUSPECTED POLYCYTHEMIA VERA, AND NORMAL CONTROLS

Total white cell count (thous. per c.mm.)	Polycythemia vera (% at level indicated)	Suspected polycythemia vera (% at level indicated)	Normal controls (% at level indicated)
0–10	11.5	3.3	4.0
11–20	11.5	23.4	18.0
21–30	23.1	20.0	20.0
31–40	23.1	13.4	24.0
41–50	3.9	13.4	16.0
51–60	7.6	10.0	4.0
61–70	11.5	3.3	0
71–80	3.9	6.6	10.0
81–90	3.9	0	0
91–100	0	6.6	0
101–110	0	0	2.0
111–120	0	0	0
121–130	0	0	2.0
131–140	0	0	0

Comparative studies of the total nucleated cells and of the total white cells are tabulated respectively in Tables 3 and 4. Study of Table 3 shows no great differences in the 3 groups of individuals as far as the total nucleated counts in the sternal marrow are concerned. The percentage ranges are comparable. In the polcythemia vera group 60% had total nucleated cells below

50,000 per c.mm. of sternal marrow as compared with 68% in the controls and 56.6% in the borderline group. The maximum recorded level in the polycythemia group was 107,000 nucleated cells per c.mm. as compared to 138,000 in the normals and 120,481 in the borderline group There is no evidence in these cases that the total number of nucleated cells per c.mm. in the sternal marrow of patients with polycythemia vera varies in any significant way from the normal range.

Study of Table 4 reveals no appreciable significant differences in the range of total white cells per c.mm. in the sternal marrow of the 3 groups. The polycythemia group and the borderline group had 73.1 and 73.5% respectively with total white counts of 50,000 or less per c.mm., of sternal marrow whereas 82% of the normal group were within this range. The maximum white count recorded in the normal group was 121,440 per c.mm. as compared with maximums of 100,000 in the borderline group and 81,320 in the polycythemia vera group. The studies tabulated here would seem to indicate that there is no relative increase in the total number of white blood cells per c.mm. of sternal marrow in polycythemia vera.

Analysis of the differential counts of the white blood cell series in the sternal marrow of the 3 groups (Table 5) reveals rather striking similarities both in regard to percentage range and average percentages. The only significant difference between the control group and the polycythemia vera group is in the percentage of segmented neutrophils. These cells are more numerous in the polycythemia group, apparently with a slight reduction in the percentage of "undifferentiated" and neutrophilic myelocytes, as compared with the normals. As might be expected, the borderline group of cases shows average values intermediate between those of the normals and the polycythemia group. These findings would seem to be in accord with the neutrophil leukocytosis so commonly found in the peripheral blood of patients with polycythemia vera.

Differential studies on the erythroid series (Table 6) reveal a moderate but definite increase in the average as well as the range of values for erythroblasts per 100 white cells counted in the polycythemia group as compared to the normals. The borderline group again occupies an intermediate position. This would appear to indicate a moderate increase in the activity of the erythroid series at the erythroblastic level in the polycythemia vera group, and to a less extent, in the borderline group. Average values for the other nucleated erythroid elements are not significantly different. In general, the total number of nucleated red cells per 100 white cells is significantly elevated in the polycythemia group as compared to the normal group, as shown by an average figure of 31.7 nucleated red cells per 100 white cells counted in the polycythemia group as compared to 15.76 in the normal group, and 21.2 in the borderline group. Further evidence of a moderate but definite increases in the activity of the erythroid series in polycythemia vera is found in Table 7, indicating a generally higher average level of reticulocytes in the sternal marrow of polycythemia vera than in that of normal individuals.

Study of the myeloid-erythroid ratio in the 3 groups (Table 8) reveals a definite rise in the proportion of erythroid cells to myeloid cells in the polycythemia vera group. In this group 56% of the patients showed myeloid-erythroid ratios of 2.9:1 or below as compared with 14.2% at this level or below in the normals and 25.8% in the borderline group. Taking 4 or 5 to 1 as the usual adult ratio, 76% of the polycythemia group are found to have ratios lower than 4.9:1, as compared with 61.3% in Group 2 and 42.6% in the normals. The range of myeloid-erythroid ratios in the normal controls was greater than in either of the other 2 groups, possibly to be attributed to the lower average ages of the group.

Megakaryocyte values could not be determined satisfactorily on smears made from oxalated marrow. Instances with extremely large numbers of megakaryocytes in the marrow have been recorded,[1] but this is not a constant finding. Increase in the platelets of the blood was not noted in any of the cases studied in Groups 1 or 2.

Discussion and Conclusions. On the basis of the study of the sternal bone marrow in these groups, it seems apparent that certain definite patterns may be found in the sternal marrow of patients with polycythemia vera: (*a*) The bone marrow as obtained from the sternum may be and frequently is entirely within the limits found in healthy normal individuals, suggesting that the bone marrow actually is entirely normal or, if hyperplasia exists, there is no variation from

TABLE 5.—ANALYSIS OF DIFFERENTIAL PERCENTAGES OF WHITE BLOOD CELLS IN POLYCYTHEMIA VERA, SUSPECTED POLYCYTHEMIA, AND NORMAL CONTROLS

	Group 1 Polycythemia vera		Group 2 Suspected polycythemia		Group 3 Normal controls	
	Average	Range	Average	Range	Average	Range
Polymorph neutrophil, segs.	44.3	22–80	36.47	18–55	31.9	13–55
Polymorph neutrophil stabs.	15.2	2–32	16.68	1–25	17.9	2–44
Polymorph neutrophil juveniles	9.2	0–22	10.71	1–23	11.8	1–21
Polymorph eosinophil	2.6	0–7	2.46	0– 8	2.9	0–15
Polymorph basophil	0.76	0– 2	0.41	0– 2	0.44	0– 2
Myelocytes, "undifferentiated"	2.5	0–17	2.22	0– 5	5.96	0–15
Myelocytes, neutrophil	3.8	0–19	3.65	0–13	8.0	0–16
Myelocytes, eosinophil	0.57	0– 2	0.78	0– 6	0.66	0– 1
Myelocytes, basophil	0.04	0– 1	0.19	0– 3	0.06	0– 1
Myeloblasts	1.2	0– 3	0.72	0– 3	1.46	0– 4
Lymphocyte, small	11.6	0–31	17.12	8–38	14.08	2–35
Lymphocyte, large	2.3	0– 9	4.81	0–20	3.36	0–11
Lymphocyte, early	0.07	0– 1	0.12	0– 1	0.02	0– 1
Monocyte	2.1	0– 6	2.84	0– 6	2.80	0–11
Monoblast	0.04	0– 1	0	0	0.02	0– 1
Plasma cell	0.31	0– 5	0.53	0– 7	0.18	0– 3

TABLE 6.—NUCLEATED RED BLOOD CELLS PER 100 WHITE CELLS COUNTED IN MARROW OF POLYCYTHEMIA VERA, SUSPECTED POLYCYTHEMIA, AND NORMAL CONTROLS

	Group 1 Polycythemia vera		Group 2 Suspected polycythemia vera		Group 3 Normal controls	
	Average	Range	Average	Range	Average	Range
Normoblasts	3.3	0–12	2.38	0– 6	2.28	0–31
Erythroblasts	28.2	1–69	18.29	5–71	13.2	3–43
Megaloblasts	0.2	0– 2	0.53	0–16	0.26	0– 2
Hemohistiocytes	0	0	0	0	0.02	0– 1

TABLE 7.—PERCENTAGE OF RETICULOCYTES IN STERNAL MARROW OF POLYCYTHEMIA VERA, SUSPECTED POLYCYTHEMIA, AND NORMAL CONTROLS

	Group 1 Polycythemia vera		Group 2 Suspected polycythemia vera		Group 3 Normal controls	
	Average	Range	Average	Range	Average	Range
Reticulocytes	3.02	0–6	2.60	0.5–10	1.87	0–6

TABLE 8.—THE MYELOID–ERYTHROID RATIO OF STERNAL MARROW IN POLYCYTHEMIA VERA, SUSPECTED POLYCYTHEMIA, AND NORMAL CONTROLS

Myeloid-erythroid ratio	Polycythemia vera (% at level indicated)	Suspected polycythemia vera (% at level indicated)	Normal controls (% at level indicated)
0–0.9:1	0	0	0
1–1.9:1	28.0	12.9	6.1
2–2.9:1	28.0	12.9	8.1
3–3.9:1	8.0	12.9	18.2
4–4.9:1	12.0	22.6	10.2
5–5.9:1	12.0	12.9	10.8
6–6.9:1	0	9.7	6.1
7–7.9:1	8.0	3.2	10.2
8 or more:1	4.0	12.9	30.3

the normal in the proportions of the cellular elements. (*b*) The bone marrow frequently shows definite variation from the normal in the proportions of cellular elements, *i. e.*, an increase in the number of nucleated red blood cells per 100 white cells counted, particularly in the number of erythroblasts, an increased number of reticulocytes, an increase in the proportion of erythroid cells to myeloid cells, and an increase in the relative numbers of segmented neutrophils. It is probable, but not a certainty, that the sternal marrow myelogram in these cases is representative of marrow cellular patterns in other locations in the body, but this can be determined only by adequate simultaneous examination of bone marrow from different locations in the body and remains a problem for further study.

Since there is no satisfactory means of determining the total volume of the bone marrow in living subjects, a clinical diagnosis of hyperplasia of the bone marrow in polycythemia vera can be made only by the demonstration of variations in the cellular morphology of the marrow suggesting hyperactivity, or by the demonstration of red bone marrow in locations where it does not normally appear in adults. The latter method is not clinically practical during the patient's lifetime. The marrow examination therefore seems to have diagnostic value in polycythemia vera only when the above-mentioned pattern (*b*) exists. This pattern, however, occurs with sufficient frequency to be of practical diagnostic value when present.

In the polycythemia vera group of 22 cases, 8 (Cases 1, 2, 4, 5, 9, 12, 14 and 15) showed a pattern believed to be characteristic of polycythemia vera when present, consisting of values of nucleated red cells of 20 or more, reticulocyte counts of 2% or higher and myeloid-erythroid ratios of 3:1 or below. In 5 of these 8 patients the nucleated red cell count per 100 white cells was above 40. In the remaining 14 cases of polycythemia vera, 1 or more components of the pattern were found in 9 cases as follows: nucleated red cell counts of 20 or more per 100 white cells were counted in 4, reticulocyte counts of 2% or higher in 4, and myeloid-erythroid ratios below 3:1 in 2 instances.

In the borderline group of 29 cases, no case presented the complete characteristic pattern, but nucleated red cell counts of 20 or more per 100 white cells counted were found in 8, reticulocytic counts of 2% or higher in 9, and myeloid-erythroid ratios of 3:1 or below in 6. In the control group of 50 cases, the complete pattern was found in 3 cases only, and in 1 of these the increased nucleated red cell count was attributed to an increase in normoblasts rather than erythroblasts, the cause for which was not apparent. In 7 other control cases, nucleated red counts of 20 or more per 100 white cells counted were found, but in only 2 of the 7 cases were counts exceeding 30 found. Reticulocyte counts of 2% or higher were found in 7 control cases, and myloid-erythroid-ratios of 3:1 or below occurred in 4 instances.

In individuals with values for hemoglobin, erythrocytes and volume of packed cells above the normal range for healthy individuals and in whom causes of secondary erythrocytosis can be excluded, the finding in the sternal marrow of a count of 20 or more nucleated red blood cells per 100 white cells, principally consisting of erythroblasts, a reticulocyte count of 2% or higher, and an increased ratio of erythroid cells to myeloid cells (myeloid-erythroid ratio of 3:1 or lower) should suggest a diagnosis of polycythemia vera. The presence of an increased percentage of segmented neutrophils is also helpful in the diagnosis. It is to be stressed, however, that the examination of the sternal marrow in polycythemia vera is a useful diagnostic supplement only, and that these variations from normal are of value when supporting polycythemic changes found in the peripheral blood.

Summary. 1. Comparative studies of marrow material obtained by sternal bone

marrow puncture in 22 cases of polycythemia vera, 29 cases of suspected polycythemia, and 50 normal individuals were made in an effort to determine whether recognizable abnormalities in the sternal marrow were present or not.

2. No significant differences were found in the total counts of nucleated cells or of white blood cells in the 3 groups.

3. Significant increases in the average values of segmented neutrophils, nucleated red blood cells, particularly erythroblasts, and reticulocytes were found in the sternal marrow of the polycythemia vera group, as compared with the normal group, with intermediate increases in the group of borderline cases.

4. The ratio of erythroid to myeloid cells was frequently increased in the polycythemia vera group.

5. A diagnosis of polycythemia vera should be suggested by a sternal bone marrow cellular pattern in which the values of nucleated red cells, principally erythroblasts, exceeds 20 per 100 white blood cells counted, in which the value of reticulocytes exceeds 2% and the value of myeloid-erythroid ratios is below 3:1 in individuals with values of hemoglobin, erythrocytes and volume of packed red cells above the upper limits of normal for healthy individuals and in whom causes of secondary erythrocytosis can be excluded. Polycythemia vera may, however, exist with entirely normal bone marrow morphology.

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REFERENCES

1. Dameshek, W., and Henstell, H. H.: The Diagnosis of Polycythemia, Ann. Int. Med., **13**, 1360, 1940.

2. Mendell, T. H., Meranze, D. R., and Meranze, T.: The Clinical Value of Sternal Bone Marrow Puncture, Ann. Int. Med., **16**, 1180, 1942.

3. Osgood, E. E., and Seaman, A. J.: The Cellular Composition of Normal Bone Marrow as Obtained by Sternal Puncture, Physiol. Rev., **24**, 46, 1944.

4. Piney, A., and Hamilton-Paterson, J. L.: Sternal Puncture, William Heinemann, Medical Book, Ltd., 1943.

5. Wintrobe, M. M.: The Normal Bone Marrow, Clinical Hematology, Phila., Lea & Febiger, p. 48, 1946.

ANEMIA AND ITS RELATION TO DIAPHRAGMATIC HERNIA

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Observations on the medical service of the Indiana University Medical Center have led to the study of anemia and diaphragmatic hernia. That anemia is a symptom of this clinical condition has been appreciated, but the relative frequency of this clinical finding has not until recently been known. The work of Murphy in 1942, reporting 47 cases of anemia in a series of 67 cases, a total of 66.7%, stimulated the study of anemia in this series.

Historical. The literature since 1920 shows a gradually increasing number of articles referable to diaphragmatic hernia and anemia. The condition of diaphragmatic hernia through the esophageal hiatus has become a clinical entity, and it is a frequent observation that a secondary anemia or history of gastro-intestinal bleeding may occur in the course of a persistent or recurrent hiatus hernia. However, the frequency with which anemia is associated, or the percentage in which it is seen in reported series of diaphragmatic hernia, has not until recently been the subject of clinical reports.

In the *Annals of Surgery* in 1918, William A. Downes[2] reported a case of a 7 year old boy with a thoracic stomach who had a red blood count of 3.4 million and a hemoglobin of 65%. There were no remarks concerning the anemia but, outside of the traumatic herniæ associated with the war surgery, this case report is one of the first which mentioned the blood count in association with this condition. In the same journal, the next year, A. L. Soresi[20] reported 3 cases, 2 traumatic, in soldiers, and 1 non-traumatic in a 19 year old girl. The statement concerning the girl was that her chief complaint was an anemia which was unexplained on the basis of visible blood loss. In the early 1920's, L. Frank[6] reported 1 case without a history of bleeding which showed a dimunition of red corpuscles and a hemoglobin of 17%. No further statement was made concerning the anemia. Portis and Portis[15] in 1920 reported 1 case of diaphragmatic hernia and in the discussion of the case reported hematemesis was also a symptom of this condition.

In 1929, E. P. Richardson[16] reported anemia in 3 of the 5 cases operated, but we do not have the total number of cases which were used to choose those which were operated upon. Segal,[19] in 1931, reported 5 cases with symptoms of anemia only, and 5 cases of gastro-intestinal symptoms also, concluding that in the symptomless case of secondary anemia it is important to look for a diaphragmatic hernia. Weitzen,[21] in 1932, reported the cases of severe anemia found in Harrington's operated series of 30. In 1933, K. D. Gardner[7] reported 22 previously published and 6 previously unpublished cases of diaphragmatic hernia with anemia. He stated that hernia was the primary factor in anemia in 8 cases and that true peptic ulcer was a complicating factor in 5. In the anemia of the other 12 cases, he allows only the supposition that the anemia was caused by the hernia. Bock, Dulin and Brooke[1] in their 10 cases of hernia with anemia substantiated the hernia as the cause of the anemia by autopsy findings in 2, and operative findings in 3, 50%. Winans,[22] in reporting the syndrome of kyphosis, gastric hernia and anemia, states that, from considera-

tion of his cases, gastric hernia must be listed and looked for as a cause of persistent hypochromic anemia. The French literature has several articles which refer to a form of diaphragmatic hernia spoken of as the anemic form. The number of instances of cases of anemia in diaphragmatic hernia, with or without the history of bleeding, are reported but the incidence is not found.

CLINICAL. The incidence of hiatus hernia has been variously reported in different series as between 2.4 and 9% of routine series of Roentgen ray examinations of the gastro-intestinal tract. Harrington found that in 5% of 100 consecutive laporotomies he was able to palpate or to put 3 to 4 fingers into the hiatal aperture, and concluded that this percentage could have a diaphragmatic hernia. The condition occurs twice as frequently in females as in males, this being correlated in all of the series of cases reported.

The etiology of hiatus hernia is a large aperture in the diaphragm which may be either congenital, traumatic or a potentially large aperture in one of the hiatal openings plus weakened musculature about the hiatus. Granting that traumatic and congenital herniæ of the diaphragm are responsible for a part of the incidence of this disease, it has been shown more frequently in recent years that the greater percentage of cases of diaphragmatic hernia occur because of a potentially large hiatal ring affected by weakened musculature and nervous or pressure influences upon this potential opening. It is an established fact that persistent irritation of the vagus causing contraction and subsequent shortening of the esophagus on a traction basis produces some of the small esophageal hiatus herniæ, especially of the recurrent type, and that increased intra-abdominal pressure as pregnancy, constipation, adiposity, or external blows on the abdomen may cause a pulsion type of hiatus hernia.

The etiology of anemia in diaphragmatic hernia is the loss of blood from the gastro-intestinal tract. In most cases, congestion or erosion of the gastric mucosa occurs proximal to the constricting ring of the hernia. Occasionally actual ulcers found in the mucosa of the herniated gut have been reported at postmortem, at operation and at gastroscopy. However, there seem to be as many cases of hernia without evidence of active bleeding as there are those which show the bleeding points. The French authors postulate the etiology of the anemia in the absence of actual bleeding points on the inability to absorb iron or on some change in the extrinsic factor responsible for a hypochromic anemia. Dyke and Dyas[3] report extraordinary response to iron therapy in their cases which would be against Goodall and Hoyt's assumption of bleeding from the gastric mucosa and more for Gardner's obscure changes giving deficient absorption of iron. Most of the literature from this country explains anemia in diaphragmatic hernia on hemorrhage and associated ulcers or slow blood loss due to a congested mucous membrane.

The clinical manifestations of diaphragmatic hernia may be protean or absent. The symptoms and clinical picture depend upon the morbid anatomy in each case. The symptoms depend upon the degree and the disturbance of function of nearby organs. There may be thoracic, abdominal, or constitutional symptoms, the latter which are on the basis of anemia, and which are occasionally associated with the disease. The symptoms are intermittent and usually not progressive. The gastro-intestinal symptoms are often vague in early and middle life but become persistent in later life, as the relaxation in the musculature about the hiatal ring increases. Gastro-intestinal symptoms vary and may simulate ulcer, gall bladder disease, cardia spasm and other similar abdominal conditions. The thoracic symptoms, when they occur, are anginal in character and may simulate true angina pectoris. The angina occurs after meals or on exertion or on lying down with a full stomach. Dyspnea, perspiration, vertigo, bradycardia and ex-

trasystoles are other symptoms referable to the thoracic group of complaints.

Considering the general symptoms of hiatus hernia, it is to be remembered that frequently this finding is incidental and not the cause of the patient's illness. This was most strikingly shown by Harrington[10] who reported cholecystitis, peptic ulcer, diverticulitis and carcinoma of the stomach or esophagus as associated conditions. However, he also reported that of the 60 cases operated in his series between 1925 and 1933, 29 had been treated for gall bladder disease with 13 having had gall bladder operations; 23 had been treated for stomach trouble; 18 had been treated for ulcer; 7 had been treated for heart disease, 4 of whom had angina pectoris; 7 had been treated for secondary anemia; 4 for intestinal obstruction and 10 had been treated for esophageal obstruction. The general symptoms of this disease are pressure in the epigastrium on eating, spasm in the esophagus causing retrosternal pain and easy vomiting which induces relief. Vague epigastric distress or substernal distress after a meal may radiate to the back or to the left shoulder. Heartburn and postprandial distress are severe and there may be pain on lying down or on stooping. The epiphrenal syndrome of von Bergmann is quite characteristic of this disease; the fullness after a heavy meal and vomiting *versus* no trouble on the taking of small amounts of food should make one consider such a diagnosis. There may be gross hematemesis and severe melena if bleeding is present and severe. The symptoms may occasionally be confused with a functional neurosis or the menopausal syndrome.

History of hemorrhage from the stomach, though it is one of the less common symptoms, is one which deserves much consideration and evaluation. Not all of the cases of hernia have hemorrhage or hematemesis as a symptom and not all of these are anemic, but, when hemorrhage is severe, exsanguination and shock may mean the immediate treatment of any bleeding peptic ulcer. With a history of hemorrhage, it is important to ascertain whether there is occult blood in the stool so that one can determine whether the anemia is due to unrecognized bleeding. Eisen,[4] in his review of the literature of esophageal hiatus hernia, states that secondary anemia associated with hematemesis or melena is occasionally present, but that the anemia may come on in intermittent attacks and symptoms of the anemia may be present at one period and absent at another time. Segal,[19] in an article reporting secondary anemia associated with diaphragmatic hernia, stated that there were over 400 articles on hiatus hernia in the literature but only approximately 10 which noted anemia as one of the symptoms or complications. Weakness, anorexia, pallor, exertional dyspnea are usually the constitutional effects produced by the secondary type of anemia seen in this hernia. The bleeding may be frank hematemesis or melena, or it may be insidious and found only by chemical analysis of the stool or gastric contents. When anemia is present, it is important to rule out associated disorders in other parts of the gastro-intestinal tract which may be of more importance than the hernia. Murphy[14] was the first to include or report cases of pernicious anemia in his series of diaphragmatic hernia. No other mention in the literature is made of the pernicious anemia, all cases reported being of a secondary hypochromic type. He does state that the gastric analysis is not a reliable test in herniæ because the tube may not be completely swallowed into the stomach and no free gastric acidity found. Also, trauma in passing the stomach tube may give a false positive Gregerson test and give an incorrect interpretation of bleeding by this method.

Reported Series. The reported series is a compilation of the cases which were found in the medical records of the Indiana University Medical Center coded as diaphragmatic hernia. There were very few cases found in comparison to the reported incidence of this condition because it is believed the incidental finding of a hiatus

hernia escaped coding where there was a more important primary diagnosis. To include as many cases as possible, the files of the Roentgen ray Department were also inspected, and cases having films reported as showing a diaphragmatic hernia were also included in the series. A total of 41 cases are reported in which the evaluation of anemia was made. There were 27 females and 14 males, which is in accord with the accepted predominance of females over males. The ages ranged from 3 weeks to 75 years. There were 3 cases under 5 years of age, 1 between 5 and 10, 2 between 10 and 20, 1 between 20 and 30, 3 between 30 and 40, 7 cases between 40 and 50, 8 between 50 and 60, 9 between 60 and 70 and 7 over 70. The average age in this series was 50.3 years.

Thirty of the cases showed herniation of the stomach alone, the other 11 cases having herniation of the stomach plus other abdominal viscera; colon, spleen, omentum, small intestine or combinations of the abdominal organs.

Thirty-four of the cases showed the herniation through the esophageal hiatus, 2 were found to be herniation through the foramen of Morgagni and 5 were due to large defects in the posterior leaf of the left diaphragm communicating with the esophageal hiatus.

Clinically, our cases have been divided into those patients who were admitted to the hospital for treatment of a known diaphragmatic hernia and those who were admitted for other causes where the hernia was an incidental finding or a secondary diagnosis. There were 28 cases admitted primarily for diaphragmatic hernia and 13 cases admitted for other causes. Because the hospital is a state institution and accepts a large number of referred cases for specific therapy, there is a higher incidence of cases admitted primarily for diaphragmatic hernia than is reported by other authors. According to other workers, there should be a higher incidence of hiatus hernia found coincidentally with other disease or as an incidental finding in routine gastro-intestinal work-ups.

In our series, the etiology was considered to be a traumatic episode, auto accident, causing a pulsion hernia in 3 patients. The etiology in 4 patients was definitely congenital in origin, all occurring in young children. The other 34 cases could not be assigned a definite etiology from a survey of the records and must be assumed to be due to an enlarged hiatus ring with or without weakened musculature about the hiatal orifice.

Thirty patients in the group under investigation had definite symptoms referable to the hiatus hernia. These were at times not the only symptoms complained of and sometimes not the most important, but in all of the 30 cases complaints referable to a diaphragmatic hernia were listed in the medical record. Eight patients had no symptoms referable to a defect in the diaphragm and the finding was only incidental during the period of hospitalization. Three cases were excluded in the evaluation of symptomatology because the history was discounted in the face of generalized advanced carcinoma of the abdomen. The hernia is found associated with many other clinical conditions and, in our series, pregnancy, gall bladder disease, asthma, hypothyroidism, peptic ulcer, hemorrhoids, coronary disease, fractured vertebræ and von Recklinghausen's disease were some of the primary diseases with the hernia as the associated condition.

Treatment of the diaphragmatic hernia was a medical management or no specific therapy in 22 of our cases; 19 were subjected to operative procedure, the surgical repair being complete in 9 and apparently successful. Three other cases have had recurrences. In 6 cases it was impossible to make a satisfactory closure of the large defect in the diaphragm, and the hernia was still present postoperatively. There was 1 postoperative mortality.

Anemia in diaphragmatic hernia is extremely variable and so statistics have been compiled in 2 categories to better evaluate a definite relationship or only an occasional association in this condition. Some authors have reported a history of

bleeding or known blood loss in their series of cases of esophageal hiatus hernia. Notable among these are Akerlund, Harrington, Jones, Sahler and the Massachusetts General Hospital series reported by Hapton and Sahler. Sahler and Hapton,[17] in the *American Journal of Roentgenology* in 1943, compiled various statistics from

TABLE 1.—HISTORY OF BLEEDING

Authors	Total cases reported	Cases with bleeding history	% showing bleeding
Akerlund	64	3	4.7
Harrington	30	8	26.6
Hedblom	397	10	2.5
Mass. Gen. Hosp.	221	28	12.6
Sahler	9	3	33.3
Jones, C. M.:			
Small	91	22	24.0
Large	37	6	16.2
I. U. Med. Center	41	8	19.5

the literature. They stated that Akerlund, in 64 cases, had 3 who vomited blood or had obvious bleeding and that this was the first correlation between hiatus hernia and bleeding. Hedblom,[11] in 1925, reported 19 cases from the Mayo Clinic series and collected 378 additional cases from the literature and, in describing the syndrome, mentioned that in the Mayo series, hematemesis was the most prominent symptom in 2 and in the cases collected from the literature, 8 complained of hematemesis. Boch, in 1929, was the first American to emphasize gastro-intestinal hemorrhages and hiatus hernia. Harrington,[9] in 1930, reported 30 cases, 8 of which had hemorrhage from the stomach. Of 91 cases with a small hernia, reported by Jones, 22 had a history of bleeding and, by the same author, 6 of 37 with large herniæ had bleeding. Sahler reported 3 cases of bleeding out of 9 herniæ found in his 100 consecutive gastro-intestinal Roentgen ray series. The Massachusetts General Hospital series of 221 cases, between 1930 and 1940, showed 12.6%, or 28 having a bleeding history. In our series of 41 cases, 8 had a positive history of hematemesis or melena which is 19.5% of the total group.

The second category is those series of cases which report anemia *per se* irrespective of the history of bleeding or blood loss. Some patients have hematemesis and do not show an anemia; some patients have hematemesis and anemia and some patients have an anemia and no symptoms of blood loss. A group of the same investigators who were shown in the above grouping are again listed as reporting the incidence of anemia. Though some of the series of cases were the same, the incidence of anemia here is different than the incidence of bleeding listed above. Ernstene,[5]

TABLE 2.—ANEMIA IN DIAPHRAGMATIC HERNIA

Author	Total cases of hernia	No. having anemia	% having anemia
Harrington	60	7	11.7
Ernstine and McGurl	59	2	3.4
Levy and Dugan	26	1	3.9
Gilbert, Dey and Rall	48	3	6.3
Held and Goldbloom	13	4	30.8
Murphy	67	47	66.7
Mass. Gen. Hosp.	221	27	12.2
I. U. Med. Center	41	11	26.8

in 1940, reviewed 59 cases of esophageal hiatus hernia and found 2 with hypochromic anemia. Levy and Duggan,[13] observing 26 cases, found only 1 with anemia. W. P. Murphy,[14] in 1942, reported a series of 67 cases, each with a blood examination; 7 cases showed a macrocytic type of anemia and 40 showed a secondary anemia, which gave 66% of his cases with a hemoglobin of less than 12 gm. or a red blood count of under 4 million. In Harrington's 60 operated cases between 1925 and 1933, 7 (11.7%) had anemia. Gilbert, Dey and Rall[8] had an anemia in 3 of their 48 cases, and Held and Goldbloom[12] showed an anemia in 4 of their 13 cases. The report of the Massachusetts General Hospital in its series of 221 cases was surveyed and 27 of that group had an anemia. In our own series there were 11 patients who had an anemia and 30 who had a normal blood count, which gave an incidence of anemia of 26.8%.

In attempting to evaluate the anemia in diaphragmatic hernia in our series of 41 cases, we first had to set a standard which could be called a normal blood count for the locale from which this group of patients had come. It was found that males with a hemoglobin above 12 gm

and a red blood count of over 4 million would have to be considered normal. For the females, a hemoglobin above 11 gm. and a red blood count above 3.5 million was considered normal. From this standard we evaluated the state of the blood in our cases and found that 30 of the patients had a normal blood count and/or hemoglobin. The 11 cases which showed an anemia were all secondary anemias, some a normochromic and some a hypochromic type. There was no evidence of a macrocytic type in any of the patients who had anemia.

TABLE 3.—SUMMARY OF CASES OF ANEMIA

Case	Sex	Age	History of bleeding	Roentgen ray findings	Hgb. (gm.)	RBC	Remarks
xL 36255	F	49	Pos.	Much of cardia stom.	9.5	3.00	Operated—repair but esoph. opening large; recurred
xL 9308	F	74	Neg.	Large hiatus hernia	10.5	2.96	Medical management
xL 64414	F	59	Neg.	Small esoph. hiatus hernia	10.5	3.20	Myxedema—medical management of hypothyroidism and obesity
xL 91661	F	57	Pos.	Thoracic stom. on r.	7.5	2.82	Suspicious evidence duod. bulb dis. on Roentgen ray
xL 95908	M	54	Pos.	Small diaph. hernia	11.0	3.43	Medical management
xR 81550	F	7	Neg.	½ stomach in r. chest	10.5	..	Surg. repair satisfactory after 2 years
xL 103953	M	60	Pos.	Large diaph. hernia of cardia	10.0	3.08	Surg. exploration; hiatus opening enlarged and stomach sutured
xL 74242	M	67	Neg.	Large diaph. hernia of cardia	9.5	2.87	Non-visualizing gall bladder on Roentgen ray
xL 65359	F	59	Neg.	Obst. antrum stom. bowel in l. thorax	9.0	3.93	Surg. repair 1942 satisfactory; recurrence 1946
xR 88891	M	2	Neg.	Herniation on absence of l. leaf diaphragm	11.0	..	Died of pneumonia and ateiectasis
xL 65702	F	48	Neg.	Stom., colon, spleen, intest.	9.5	3.00	Operated—defect too large to close; died 3 days post-opr.

In the 11 cases which showed an anemia, 7 were females and 4 were males. Four of the patients had a positive history of hematemesis or melena and these were equally divided between males and females. The other 7 had had no recorded history of any gastro-intestinal hemorrhage or bleeding. There was no correlation between the hernial contents or the size of the hernia and whether the patients were anemic; there were equally as many small and large herniæ in the normal group as among the 11 patients who presented the anemia.

Though the 11 cases of anemia are too few to make any positive conclusions about treatment, from the follow-up, it was found that in the one satisfactory surgical repair the anemia disappeared. It is still present or has returned in the 2 postoperative cases that had recurrences. In those cases treated medically, the anemia improved in several cases and was still present in 1 who had known hematemesis. There was no follow-up in another patient. From the literature it is reported that the anemia of diaphragmatic hernia does respond well to iron therapy.[18]

Summary. A review of the clinical entity diaphragmatic hernia, and the secondary anemia which may be found with it, has been presented. Forty-one more cases of hiatus hernia are listed. In this series 11 patients showed a secondary anemia, a percentage of 26.8. This incidence of anemia is not as high as some reported series, but is higher than a number of other series. It tends to substantiate the fact that anemia in diaphragmatic hernia is often found as part of the disease and that it does not have to be associated with a history of gastro-intestinal bleeding.

REFERENCES

1. Bock, A. V., Dulin, J. W., and Brooke, P. A.: New England Jour. Med., **209**, 615, 1933.
2. Downes, W. A.: Ann. Surg., **67**, 748, 1918.
3. Dyke, S. C., and Dyas, G. E.: Lancet, **1**, 119, 1940.
4. Eisen, D.: Canad. Med. Assn. J., **39**, 207, 1938.
5. Ernstene, A. C., and McGurl, F. J.: Cleveland Clin. Quart., **7**, 209, 1940.
6. Frank, L.: Ann. Surg., **71**, 280, 1920.
7. Gardner, K. D.: Am. J. Med. Sci., **185**, 561, 1933.
8. Gilbert, N. C., Dey, F. L., and Rall, J. E.: J. Am. Med Assn., **132**, 132, 1946.
9. Harrington, S. W.: Surg., Gynec. and Obst., **51**, 504, 1930.
10. Harrington, S. W.: J. Am. Med. Assn., **101**, 987, 1933.
11. Hedblom, C. A.: J. Am. Med. Assn., **85**, 947, 1925.
12. Held, I. W., and Goldbloom, A. A.: Rev. Gastro-enterol., **3**, 291, 1936.
13. Levy, M. D., and Duggan, L. B.: South. Med. J., **34**, 351 1941.
14. Murphy, W. P.: Bull. New England Med. Center, **4**, 7, 1942.
15. Portis, M. M., and Portis, S. A.: J. Am. Med. Assn., **75**, 1262, 1920.
16. Richardson, E. P.: Surg., Gynec. and Obst., **49**, 129, 1929.
17. Sahler, O. D., and Hapton, A. O.: Am. J. Roentgenol. and Rad. Ther., **49**, 433, 1943.
18. Schiro, H. S., and Benjamin, J. E.: Ohio State Med. J., **36**, 164, 1940.
19. Segal, H. L.: New York State J. Med., **31**, 692, 1931.
20. Soresi, A. L.: Ann. Surg., **69**, 254, 1919.
21. Weitzen, M.: Am. J. Roentgenol., **28**, 808, 1932.
22. Winans, H M.: Texas State J. Med., **34**, 412, 1938.

PROLONGED INCUBATION PERIOD AS AN EPIDEMIOLOGIC PRINCIPLE

INFECTIOUS HEPATITIS AND HOMOLOGOUS SERUM JAUNDICE

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IN clinical medicine "the case" begins with the onset of sickness and terminates when the patient is well again; in epidemiology "the case" begins when the patient receives the infectious agent and lasts as long as he is infectious. In certain diseases the clinical and epidemiologic cases may closely coincide but in many they are widely or even totally divergent. In some diseases the epidemiologic case begins long before and long outlasts the clinical case and in others the majority of infected and infectious individuals never have any sickness at all—subclinical infection or healthy carriage.[8] Incubation periods have been judged from intervals of time between adjacent cases or have been established from repeated observation of definite intervals elapsing between exposure to known sources of infection and the onset of disease. In many instances, they have been more precisely determined by deliberate or experimental inoculation with the disease agent. In general, incubation periods have been interpreted as representing the interval between exposure to infection and onset of disease. For example, from an earlier study of the intervals between multiple cases in families, it was concluded that in poliomyelitis "the search for the source of the infection should be centered at about the 14th day before the onset of acute symptoms."[3] It subsequently became clear that this conclusion should be amended when it was found that the incubation period—and disease—was not always initiated at the time of exposure to the infectious agent but at times by some predisposing event having an effect on the host which initiated the infectious process in an individual already harboring the virus.[6] More precisely, it may be said that "predisposing causes" may convert healthy carriage into incubation. A clear example is seen in bulbar poliomyelitis following tonsillectomy. In meningitis there are many indications that incubation may be most often initiated by some "predisposing" factor in persons already carrying the organism.

The mechanism involved in incubation periods is not known and since there are no signs or symptoms, it lends itself poorly to study. Yet, in some diseases its time factor is very precisely defined, in others this may vary within wide limits, and in some it may be definitely altered by certain special circumstances, as, for example, the prolonged incubation period under the protective influence of immune serum or other influences which "prepare" the host in the direction of resistance to inoculation with virus.[2]

PROLONGATION OF INCUBATION PERIODS. Sternberg and Reed[37] reported an early and interesting example of prolonged incubation period. From experiments on the protective effect of vaccinia immune serum against vaccination in monkeys, they concluded that the appearance of lesions is retarded as long as "there is present in the system of the vaccinated animal certain antitoxic substances contained in the serum, and that when these substances have been eliminated from the body, the process proceeds to a successful though somewhat modified course of development." Their protocols indicate that there was a retardation in appearance of the vaccinia reaction of about the same order as that in the other examples of prolongation of incubation period to be cited.

In the conduct of neutralization tests in poliomyelitis it has been observed that animals inoculated with mixtures of virus and non-immune serum develop the experimental disease—as do animals inoculated with virus alone—after an incubation period not exceeding 14 days. Inoculation with immune serum-virus mixtures usually affords complete protection. Exceptionally the disease develops after longer incubation periods (Aycock and Luther).[7] In the course of a study of various factors involved in the neutralization test, Schaffer and Muckenfuss[35] reported data on the incubation periods in a large number of tests done with a single strain of poliomyelitis virus and the same pool of human convalescent serum. The average incubation period following inoculation with virus and non-immune serum was 8.3 days and with virus-immune serum 14.3 days —a prolongation of 72% (Chart 1).

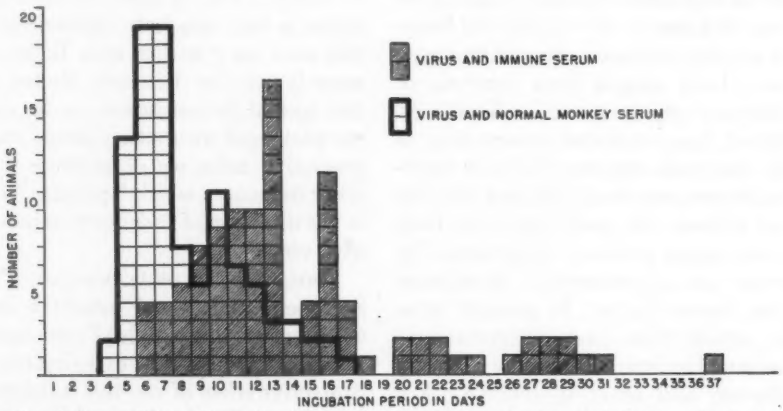
VIRUS AND IMMUNE SERUM
VIRUS AND NORMAL MONKEY SERUM
NUMBER OF ANIMALS
20
15
10
5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
INCUBATION PERIOD IN DAYS

CHART 1.—Poliomyelitis. Neutralization tests showing incubation periods in animals inoculated with mixtures of virus and immune serum and mixtures of virus and normal monkey serum. Average prolongation with immune serum—8.3 days (72%).[35]

Data relating to the average survival time (incubation period in the sense that death of the inoculated animal is the "uniformly recognizable sign of the disease") in mice employed in neutralization tests with normal human γ-globulin and influenza A virus PR 8, by Miss Julia Sullivan in the laboratory of the John F. Enders* have kindly been made available. In titrations, usually in dilutions of $\frac{1}{100}$ to $\frac{1}{800}$ of γ-globulin, average survival time of mice injected with dilutions of $\frac{1}{800}$ of globulin (81.9% succumbing), was 6.2 days, and with $\frac{1}{100}$ dilutions (with only 4.5% succumbing), 8.7 days. Thus the prolongation of incubation period as a result of the protection of γ-globulin was (within the range tested) 42% (Table 1).

Duncan, Christian, Stokes, Rexer, Nicholson and Edgar[14] have observed prolongation of the incubation period by the administration of γ-globulin to patients already in the incubation period of homologous serum jaundice. Their data comprise 52 out of 4780 battle casualty patients (with no history of jaundice) who had received blood or plasma or both at the time of injury and who developed hepatitis within 5 months, but after 10 days from the date of administration of γ-globulin. Of these patients 2406 received γ-globulin; 29 developed hepatitis, the mean interval from the original transfusion to the development of hepatitis being 103 days, and from administration of γ-globulin, 53 days. Of the 2374 alternate

* These studies were carried out by Dr. John F. Enders of the Department of Bacteriology of the Harvard Medical School, with γ-globulin prepared by the Department of Physical Chemistry, Harvard Medical School, from blood collected by the American Red Cross, under a contract recommended by the Committee on Medical Research and Development and Harvard University.

patients used as controls, 23 developed hepatitis after a mean interval of 84 days from original transfusion and 34 days from the day on which they were paired with patients receiving γ-globulin and designated as controls. (The average intervals between the time the 2 groups received blood or plasma and the time they were injected with globulin or designated as controls, were "almost identical.")

TABLE 1.—PROLONGATION OF INCUBATION PERIOD—IMMUNOLOGIC PROTECTION: NORMAL γ-GLOBULIN AGAINST INFLUENZA A VIRUS PR 8

Globulin dilution	Mice surviving	Mice dying	% dying	Av. days survival in mice dying
1–800	58	260	81.9	6.2
1–400	80	232	74.3	7.1
1–200	203	111	35.3	7.7
1–100	298	14	4.5	8.7

Thus, as concluded by these authors, the administration of a single dose of 10 cc. of γ-globulin (on the average on the 50th day of an average incubation period in untreated controls of 84 days) retarded the onset of the acute phase of hepatitis by an average period of 19 days (23%). However, since γ-globulin was administered late in the incubation period, a more exact expression of the effect of γ-globulin would be the degree of prolongation of that part of the incubation period represented by the interval between the date of administration of globulin and onset of the disease in controls—a prolonged "incubation" of 55% over that of the controls.[14]

Prolongation of incubation periods does not appear to be a simple "coating of virus with antibody," whereby the onset of symptoms is delayed for a constant period. Prolongation by immune serum is more nearly proportionate to the length of the incubation periods of the several diseases, or (in the case of homologous serum jaundice treated in mid-incubation period with γ-globulin) to that part of the incubation affected by immune serum. Thus, actual prolongations of 6, 2.5 and 19 days in the 3 examples cited represent degrees of prolongation of 72%, 42% and 55%. The disease ensuing after a prolonged incubation is in no way distinguishable—in clinical manifestations, severity, or mortality—from that following shorter incubation periods. This would indicate that the action of the immune serum is restricted to prevention of disease entirely or to prolongation of incubation. This is true in experimental poliomyelitis, apparently so in the γ-globulin influenza tests, as well as in homologous serum jaundice where γ-globulin was administered in the incubation period of the disease. In other words, prolonged incubation period is the only effect produced on disease resulting from inoculation of virus "partly neutralized" by immune serum. In this connection a comparison with modification of measles with immune serum would be of interest.

INFECTIOUS HEPATITIS AND HOMOLOGOUS SERUM JAUNDICE. The striking difference between infectious hepatitis and homologous serum jaundice (in jaundice occurring spontaneously and following injection of human serum), and the main basis for the question of there being 2 separate entities, is the difference in incubation periods—striking in terms of time alone—but in proportion to the length of the incubation period, not greatly in excess of the prolongation of incubation periods obtained in other diseases when virus is "partially neutralized" with immune serum. There are epidemiologic grounds for the assumption that icterogenic serum is derived from persons either in the incubation, acute or convalescent stage of infectious hepatitis or from persons subclinically infected with it. On the other hand, there are good epdiemiologic grounds for assuming that the virus causing homologous serum jaundice is not a different etiologic agent from that causing infectious hepatitis. For instance, there is no evidence for the

existence of a spontaneous disease caused by a homologous serum jaundice virus which could serve as the "virus reservoir" for homologous serum jaundice. Actually, it would seem to be somewhat of an anomaly to say that many persons harbor such a virus without its ever causing disease in them and yet, when their blood serum is injected into others, the disease is readily produced. It would appear much more logical to infer that the "virus reservoir" for homologous serum jaundice is individuals who harbor the virus of infectious hepatitis—one evidence of which is the occurrence of the spontaneous disease in them, and another that blood serum from them produce a disease indistinguishable from homologous serum jaundice when inoculated into others—even as to incubation period.

In this paper, the possible relationship of prolonged incubation resulting from inoculation of virus in the presence of immune serum to the epidemiology of infectious hepatitis and homologous serum jaundice will be discussed.

The question is raised whether the shorter incubation in infectious hepatitis and the longer incubation in homologous serum jaundice may not be the result of infection, on the one hand by virus free of admixture with serum, and on the other hand, by the same virus partially neutralized by admixture with serum of donor, recipient, or both. To shed some light on this question, data have been collected from the literature on both infectious hepatitis and homologous serum jaundice where incubation periods are given in cases following inoculation (or infection) under circumstances which imply either serum-virus mixtures or serum-free virus.

The first category comprises cases of both infectious hepatitis and homologous serum jaundice known to have been transmitted by virus supposedly free of admixture with serum; and the second, cases of both diseases in which transmission was accomplished with parenteral injection of serum in which virus was present. Among the former are experimental transmissions of each disease to volunteers by intranasal or oral administration of nasal washings or feces, and cases contracted from cases of the same disease by direct contact. The latter comprise only cases of both diseases from whom the disease was transmitted to others by parenteral injection of blood or serum. Cases of homologous serum jaundice in the usual sense—contracted following inoculation of serum or plasma from supposedly normal individuals (or of unknown origin)—are not included.

For comparison of the different categories of cases from the point of view of prolongation of incubation periods, the data are restricted to instances where the type of disease from which the infectious agent was obtained, the manner by which it was transmitted, and incubation periods, were known. This requirement limits many of the observations used to transmission experiments and, because of the fact that only human volunteers can be used, these are not numerous.[9–13,16–18,20,22,23,25–34,38,39] Even though the numbers are small, however, there is a consistency in the results of the comparisons which suggests that the findings are valid.

Of 82 cases of infectious hepatitis, in 60 the disease is known to have been transmitted intranasally, orally, or by contact, with an average incubation period of 28.4 days; while in 22 cases in which transmission was by the parenteral injection of serum from other cases of infectious hepatitis, the average incubation period was 61.6 days—a prolongation of 116%. Of 17 cases of homologous serum jaundice, in 10 transmission was by intranasal or oral administration of material from cases diagnosed as homologous serum jaundice, with an average incubation period of 46.1 days; while in 7 cases following parenteral injection, it was 87.8 days—a prolongation of 90.4%. (Incubation periods by weeks are shown in Chart 2.) It may be stated that in 3 of the cases of homologous serum jaundice used here, transmission occurred by contact under circumstances where the presence of infectious hepatitis could be satisfactorily ruled out.

The incubation periods of 38, 43 and 49 days are the longest possible incubation periods, but the circumstances are such that they all might have been shorter. In both infectious hepatitis and homologous serum jaundice where transmission was accomplished by intranasal, oral or contact infection, the incubation periods tended to be that which is generally recognized as that of infectious hepatitis. When either disease was transmitted by parenteral injection of serum from the same type of case, the incubation period was that which is regarded as characteristic of homologous serum jaundice (following injection of supposedly normal serum).

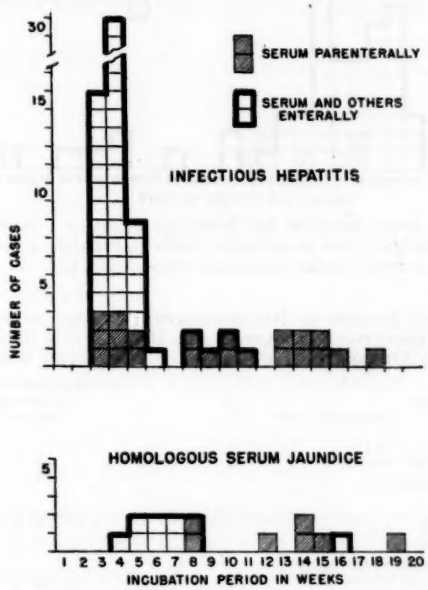

CHART 2.—Infectious hepatitis and homologous serum jaundice. Incubation periods following inoculation of serum and other virus containing material enterally, and inoculation of serum containing virus parenterally. Prolongation incubation periods with parenteral serum of 116% and 90.4%.

It thus appears that the difference in the incubation periods of infectious hepatitis and homologous serum jaundice might be accounted for largely by differences in the manner of transmission of the 2 diseases. Prolonged incubation period resulting from admixture of virus with serum containing antibodies is suggested as a probable explanation for the longer incubation period in homologous serum jaundice.* The degree of prolongation of incubation period, as between infectious hepatitis and homologous serum jaundice, is greater than that observed in any of the other examples of prolonged incubation periods induced by admixture of virus with immune serum. In all these, however, prolongation was induced either by *in vitro* mixture of virus and serum or injection of virus into hosts protected by immune serum. That prolongation in incubation period may be additive depending in part on antibodies at the source of virus (in the serum of the donor patient), admixture *in vitro* (inclusion of immune serum in pools) or antibodies in the recipient host, is suggested by the observation

* In most of the reports from which data have been collected, there is not sufficient information to make a precise analysis of the possibility that "prolongation of incubation periods" may be the result of retarded recognition resulting from modification of the disease from serum. Study of the various reports, however, does not give any indication that the thresholds of recognition of the 2 types of cases are different.

cited that when individuals in the already prolonged incubation period of homologous serum jaundice are injected with γ-globulin, the incubation period is still further prolonged. Fox[19] observed wide variation in the frequency of icterus following yellow fever vaccination, with known icterogenic lots of the vaccine in hepatitis and homologous serum jaundice combined, where the virus used was obtained from nasal washings or feces, or where the disease was naturally transmitted by contact, intranasal or oral inoculation of serum-free virus, the average incubation period was 29.9 days. In 8 cases where blood serum from the donor

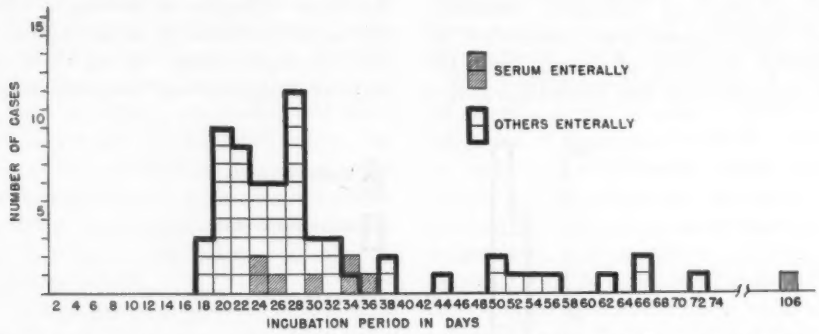

CHART 3.—Homologous serum jaundice and infectious hepatitis. Incubation periods intranasal or oral inoculation with serum-free virus as compared with intranasal or oral inoculation of serum containing virus. Prolongation with serum containing virus of 29.7%.

TABLE 2.—INCUBATION PERIODS IN NEUTRALIZATION TESTS AND IN CERTAIN CATEGORIES OF CASES OF INFECTIOUS HEPATITIS AND HOMOLOGOUS SERUM JAUNDICE—SHOWING DEGREE OF PROLONGATION ASSOCIATED WITH SERUM

Materials used	Average days incubation period	% prolongation
Poliomyelitis:		
Normal monkey serum	8.3	
Human convalescent	14.3	72.2
Influenza:		
$\frac{1}{800}$ γ-globulin	6.2	
$\frac{1}{100}$ γ-globulin	8.7	40.3
Homologous serum jaundice:		
Controls	34.1	
γ-globulin	52.8	54.8
Infectious hepatitis:		
Nasal washings, feces, or serum—enterally	28.4	
Serum—parenterally	61.6	116.9
Homologous serum jaundice:		
Nasal washings, feces, or serum—enterally	46.1	
Serum—parenterally	87.8	90.4
Infectious hepatitis and homologous serum jaundice:		
Nasal washings or feces—enterally	29.9	
Serum—enterally	38.8	29.7

similarly vaccinated localities, and in households within these localities. One possible explanation for this was preëxisting immunity to the icterogenic agent contained in the vaccine, presumably resulting from previous infection with infectious hepatitis. In this connection, it is of interest that in 62 cases of infectious hepatitis and homologous serum jaundice combined, where the virus used was obtained from nasal washings or feces, or where the disease was naturally transmitted by contact, intranasal or oral inoculation of serum-free virus, the average incubation period was 29.9 days. In 8 cases where blood serum from the donor patient was employed for intranasal or oral administration, the average incubation period was 38.8 days—a prolongation of 29.7% (Chart 3). There would, therefore, seem to be a possibility that admixture of serum from the donor patient (presumably containing antibodies) might account in part for the prolongation of

incubation periods in homologous serum jaundice over that of infectious hepatitis.

Comparisons of incubation periods with the degree of prolongation in all the different categories used in this study are summarized in Table 2. It may be of some interest to note here that when known non-immune animals were injected with supposedly serum-free virus mixed with known immune serum (poliomyelitis neutralization), the degree of prolongation was 72%. In infectious hepatitis and homologous serum jaundice, the degree of prolongation in cases where the virus was contained in serum from the donor was 29.7% more than in cases where the source of virus was presumably serum free (nasal washings or feces). If these 2 prolongations, namely, in poliomyelitis neutralization (72%) and virus hepatitis (29%), are added, they closely approach the prolongation of incubation period in homologous serum jaundice, in general, over that of infectious hepatitis (101%) (Chart 4). This would suggest that there may be antibodies both in the serum of the donor and recipient patients in homologous serum jaundice; while in naturally occurring infectious hepatitis (in non-immune persons), there may be no admixture of virus with antibodies in either.

The question of there being separate etiologic agents, apparently raised in the first place on the basis of the difference in incubation periods, had led to studies looking for other differences. Some are contradictory; others are based on small series of observations, and in still others the conclusion that there are 2 etiologic agents appears to be a matter of interpretation.

The chief differences in the 2 types of jaundice which have been emphasized, are differences in clinical manifestations, lack of cross-immunity, and the absence of contagiousness in homologous serum jaundice. The voluminous literature on these 3 points will not be reviewed here, but it may be pointed out that the weight of opinion seems to be that the 2 types of jaundice are clinically and pathologically identical. The observation of such manifestations as arthritis and skin rashes, reported as characteristic of homologous serum jaundice, has likewise been reported in infectious hepatitis; and, in general, it may be said that no clinical manifestations as yet have been established as pathog-

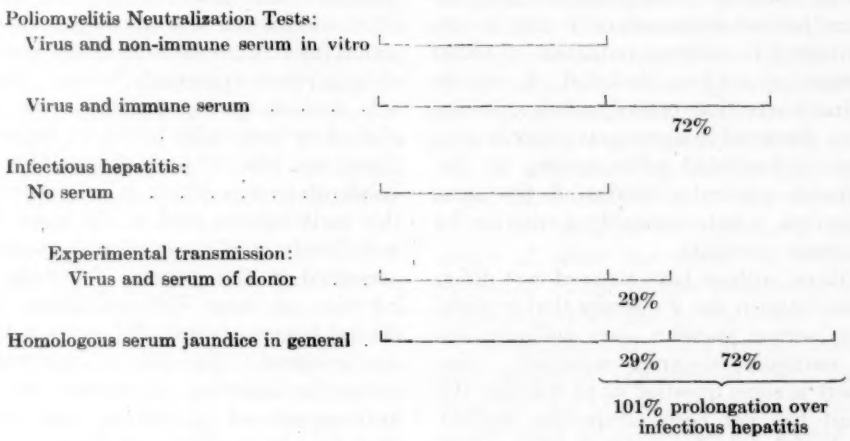

CHART 4.—Theoretical explanation of prolongation of incubation period of homologous serum jaundice over infectious hepatitis.

nomonic of either of the 2 types of jaundice.[21,24]

Knowledge of immunity in the 2 types of icterus is extremely limited because of the fact that observations have been confined to human studies. We believe that there is not yet sufficient information to establish the 2 diseases as being due to immunologically distinct agents. Too few immunologic studies have been done to rule out the possibility that lack of cross-immunity may be due to different strains of the same virus which will produce either infectious hepatitis or homologous serum jaundice. The possibility that the same lack of cross-immunity may be encountered in different outbreaks of either disease has not been excluded. It may be pointed out that cross-immunity, as has been discussed in a previous paper in relation to bacteria,[4] while serving to distinguish particular strains of the same organism, is not necessarily a criterion for etiologic specificity.

Many authors have stressed as a difference between the 2 diseases that homologous serum jaundice does not give rise to naturally occurring secondary cases. There is some question as to whether this point has yet been adequately studied. As has already been stated, "the search for the source of infection should be centered at a time preceding onset of symptoms by the length of the incubation period." With the existing conviction as to a difference in the incubation periods of the 2 types of hepatitis, it is not unlikely that secondary cases of homologous serum jaundice would be sought for at a longer interval and that cases actually occurring after a shorter interval following exposure to previous cases might well have been designated as infectious hepatitis and assigned, not to a relatively recent exposure to other cases of homologous serum jaundice, but to some supposed exposure to infectious hepatitis. It is true that relatively few cases of long incubation period infectious hepatitis and short incubation period homologous serum jaundice have been recorded. This might be accounted for in part by the use of incubation periods as the basis of differential diagnosis in many cases of otherwise indistinguishable jaundice. Finally, it is possible that there may be an actual difference in contagiousness. In infectious hepatitis, the infectious agent entering through the mouth or nose might be contagious for example, throughout its incubation period; while in homologous serum jaundice, the virus, due to the manner of inoculation, might not appear on external surfaces of the body until later or not at all.

Findlay and MacCallum,[15] in 1938, had suggested that jaundice following yellow fever vaccination was caused by virus in serum (used in preparation of the vaccine) obtained from apparently healthy persons who were in reality suffering from subclinical or very mild infectious hepatitis. Shank and Mirick[36] have observed an outbreak of icterus which is in support of this early opinion and at the same time well illustrates all the epidemiologic points presented in this paper. Following the injection of some 750 individuals with diluted human plasma, 267 cases of jaundice occurred. The average interval between the injection of plasma and the development of symptoms was about 70 days. In an equal number of persons working in the same establishment who did not receive plasma, 14 verified and probably 8 other cases of spontaneous jaundice occurred late in the outbreak. The average interval between the date of inoculation of plasma and the development of jaundice in the uninoculated or "control" group was about 100 days. No cases of spontaneous jaundice had occurred in the group during the preceding 3 years. Thus, the average interval between the primary cases (following inoculation with with plasma) and the secondary cases in the uninoculated group was about 30 days —a period coinciding with the incubation period of infectious hepatitis.

In institutional outbreaks of measles and German measles previously reported,[1,5] in each within a period of 8 weeks the primary case and 4 secondary waves of

measles occurred and in German measles the primary case and 2 secondary waves occurred. The average interval between waves in measles was 11.2 days and between waves in the German measles outbreak, 18.6 days. In these 2 frequently confused diseases the intervals between waves of cases in institutional outbreaks are a distinguishing feature.

It would therefore appear, by analogy somewhat in reverse, that in the outbreak of jaundice observed by Shank and Mirick the cases in the secondary wave (spontaneously occurring jaundice) correspond to the usual definition of infectious hepatitis. But since the source of infection for this wave of cases apparently was a preceding wave of cases of homologous serum jaundice, it is indicated that the long incubation period resulted from parenteral injection of serum and the short incubation from spontaneous infection presumably with serum-free virus by contact.

Summary and Conclusions. In neutralization and protection tests in a number of virus infections having different incubation periods, a portion of the animals inoculated with mixtures of virus and immune serum develop the disease following incubation periods that are longer than those resulting from inoculation with virus and non-immune serum. Prolongation is proportionate to the length of the incubation periods of each of the several diseases. The degree of prolongation of the incubation period of homologous serum jaundice over that of infectious hepatitis is of the order of that observed in other neutralization or protection tests.

In the light of this epidemiologic principle, it is suggested that the difference in the incubation periods of infectious hepatitis and homologous serum jaundice is due to differences in the manner of transmission of a single virus which involve admixture of virus with antibodies.

The shorter incubation period, generally regarded as characteristic of infectious hepatitis, occurs when transmission of either of the 2 types of jaundice is accomplished by intranasal or oral administration, or by contact (serum-free virus). The longer incubation period, regarded as characteristic of homologous serum jaundice, follows the transmission of either of the types of jaundice by the injection of serum containing virus. It is postulated that the prolongation of the incubation period of homologous serum jaundice over that of infectious hepatitis is the result of partial neutralization of the virus by admixture with serum containing antibodies of either the donor or recipient patient or by the *in vitro* action of serum containing antibodies on the virus.

The establishment of naturally occurring infectious hepatitis as the "virus reservoir" for homologous serum jaundice would serve to emphasize the extent of this "reservoir" and the importance of the study of methods for insuring sterility. This leads to the suggestion that, until a method of insuring sterility is found, in the administration of serum-containing products these dangers are always present; consequently the indications for their use must be important.

REFERENCES

1. Aycock, W. L.: Am. J. Med. Sci., **204**, 455, 1942.
2. Aycock, W. L., and Curley, F. J.: Variolations as a Principle, Am. J. Med. Sci. (in press).
3. Aycock, W. L., and Eaton, P.: Am. J. Hyg., **5**, 724, 1925.
4. Aycock, W. L., and Foley, G. E.: Am. J. Med. Sci., **211**, 350, 1946.
5. Aycock, W. L., and Ingalls, T. H.: Am. J. Med. Sci., **212**, 366, 1946.
6. Aycock, W. L., and Luther, E. H.: New England J. Med., **200**, 164, 1929.
7. Aycock, W. L., and Luther, E. H.: J. Prev. Med., **3**, 103, 1929.
8. Aycock, W. L., and Russell, F. F.: Am. J. Med. Sci., **206**, 399, 1943.
9. Beeson, P. B.: J. Am. Med. Assn., **121**, 1332, 1943.
10. Booth, W. K., and Okell, C. C.: Epidemic Catarrhal Jaundice, Pub. Health, London, **41**, 287, 1928.
11. Cameron, J. D. S.: Quart. J. Med., **12**, 139, 1943.
12. Darmady, E. M., and Hardwick, C.: Lancet, **2**, 106, 1945.

13. Droller, H.: Brit. Med. J., **1**, 623, 1945.
14. Duncan, G. G., Christian, M. A., Stokes, J., Rexer, W. J., Nicholson, J. J., and Edgar, A.: Am. J. Med. Sci., **213**, 53, 1947.
15. Findlay, G. M., and MacCallum, F. O.: Proc. Roy. Soc. Med., **31**, 799, 1938.
16. Findlay, G. M., and Martin, N. H.: Lancet, **1**, 678, 1943.
17. Findlay, G. M., Martin, N. H., and Mitchell, J. B.: Lancet, **2**, 301, 1944; **2**, 340, 1944; **2**, 365, 1944.
18. Findlay, G. M., and Wilcox, R. R.: Lancet, **1**, 212, 1945.
19. Fox, J. P., Manso, C., Penna, H. A., and Para, M.: Am. J. Hyg., **36**, 68, 1942.
20. Havens, W. P., Jr., Ward, R., Drill, V. A., and Paul, J. R.: Proc. Soc. Exp. Biol. and Med., **57**, 206, 1944.
21. Lucké, B., and Mallory, T.: Am. J. Path., **22**, 867, 1946.
22. MacCallum, F. O., and Bauer, D. J.: Lancet, **1**, 622, 1944.
23. MacCallum, F. O., and Bradley, W. H.: Lancet, **2**, 228, 1944.
24. Mallory, T. B.: New England J. Med., **236**, 438, 1947.
25. Ministry of Health Memorandum: Homologous Serum Jaundice, Lancet, **1**, 83, 1943.
26. Morgan, H. V., and Williamson, D. A. J.: Brit. Med. J., **1**, 750, 1943.
27. Neefe, J. R., and Stokes, J.: J. Am. Med. Assn., **128**, 1063, 1945.
28. Neefe, J. R., Stokes, J., Baty, J. B., and Reinhold, J. C.: J. Am. Med. Assn., **128**, 1076, 1945.
29. Neefe, J. R., Stokes, J., and Reinhold, J. G.: Am. J. Med. Sci., **210**, 29, 1945.
30. Neefe, J. R., Stokes, J., Reinhold, J. G., and Lukens, F. D. W.: J. Clin. Invest., **23**, 836, 1944.
31. Oliphant, J. W.: U. S. P. H. Report, **59**, 1614, 1944.
32. Oliphant, J. W., and Hollanaender, A.: U. S. P. H. Report, **61**, 598, 1946.
33. Paul, J. R., Havens, W. P., Jr., Sabin, A. B., and Philip, C. B.: J. Am. Med. Assn , **128**, 911, 1945.
34. Rennie, J. B., and Fraser, T. N.: Bull. Hyg., **21**, 647, 1946.
35. Schaeffer, M., and Muckenfuss, R. S.: Experimental Poliomyelitis, Nat. Found. for Inf. Paralysis, p. 136, 1940.
36. Shank, R. E., and Mirick, G.: Personal communication.
37. Sternberg, G. M., and Reed, W.: Trans. Assn. Am. Phys., **10**, 57, 1895.
38. Van Langenberg, E. R.: Lancet, **1**, 244, 1943.

CARONAMIDE, A COMPOUND THAT INHIBITS PENICILLIN EXCRETION BY THE RENAL TUBULES, APPLIED TO THE TREATMENT OF SUBACUTE BACTERIAL ENDOCARDITIS*

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A NEW compound, caronamide,[26]† has been described as orally effective to elevate the plasma concentrations of penicillin. Large losses of penicillin in the urine are known to occur, and since it is now established that approximately 80% of the loss is through the renal tubules,[9,21] an agent capable of inhibiting the tubular excretion of penicillin might be expected to enhance the levels of penicillin in the plasma. These effects have been observed to follow the administration of diodrast,[22] para-aminohippuric acid[4,7,9] and benzoic acid.[12,25] Diodrast and para-aminohippuric acid have been employed in the treatment of subacute bacterial endocarditis,[1,18,20,24] but the necessity of administering large amounts of either substance by constant intravenous infusion has limited their usefulness.

It has been hypothesized that the physiologic and reversible inhibition of the penicillin transport mechanism is due to a "substrate competition between penicillin which is excreted by the tubules, and 4′-carboxy-phenylmethanesulfonanilide, which is essentially refractory to excretion by the transport mechanism."[2]

In preliminary clinical investigations,[15] penicillin plasma concentrations were enhanced from 3 to 7 fold by the oral administration of caronamide, and no serious toxic manifestations were observed. Accordingly, we felt justified in applying caronamide to the treatment of a patient with subacute bacterial endocarditis due to a strain of *Streptococcus viridans* resistant to penicillin and to streptomycin.

Case Abstract. The patient, M. C., was a white female aged 51 years. The diagnosis of subacute bacterial endocarditis was established on Aug. 1, 1946, at which time the blood culture was positive and the heart murmurs were observed to have changed in character.

As shown in Table 1, the patient had received 4 courses of antibiotic therapy, 2 of penicillin and 2 of streptomycin prior to this study. The first course totalling 14,400,000 units of penicillin was given over a period of 29 days administered by intramuscular injection every 3 hours in a daily dose of 540,000 units. Following this course of therapy, the blood culture became negative but signs of relapse were observed on October 14, and blood cultures remained positive until the second course of penicillin therapy began on October 29. A daily dose of 750, 000 units of penicillin was given for

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The penicillin used in this investigation was supplied through the courtesy of Charles Pfizer & Co., Inc. Caronemide was supplied through the courtesy of Sharp & Dohme, Inc.

† Since we have had the benefit of knowing by personal communication about many of the investigations on caronamide that are now in progress, the Bibliography contains a number of references to unpublished data.

50 days, the total amount being 37,500,000 units. Penicillin was administered by intramuscular injection every 2 hours. Again the blood cultures became negative and the disease appeared to be in remission but renewed activity of the disease and a positive blood culture appeared on Dec. 31, 1946. The original sensitivity of the strain of *Streptococcus viridans* was 0.03 unit of penicillin per cc., but under therapy this decreased to 0.5 unit per cc. Accordingly, a change to streptomycin was made on January 13. A daily dose of 3 gm. was given by intermittent intramuscular injections every 4 hours. Treatment was continued for 15 days and a total of 45 gm. of streptomycin was administered. When streptomycin therapy was initiated, the organism was sensitive to 0.5 unit per cc. but after treatment 5 units per cc. were needed to inhibit growth. A second course of 24 gm. of streptomycin was given over a period of 8 days. At the conclusion of the fourth course of treatment and the second course of streptomycin, the organism was found to have increased its resistance to streptomycin by 20,000 fold, 10,000 units per cc. being required to inhibit growth and the organism was resistant to 0.5 unit per cc. of amorphous penicillin and fraction G and X. After 4 courses of treatment the blood cultures remained positive and the bacterial endocarditis was active as indicated by fever, embolic phenomena and a deterioration of the general state of health.

Since the resistance of the infecting organism in this instance appeared to require high penicillin plasma concentrations, it seemed justifiable to use caronamide as an adjunct to intensive penicillin therapy. In Chart 1 the details of this patient's fifth course of antibiotic therapy are presented. The record of rectal temperatures suggests the severity of the illness under treatment; weight loss, anorexia and asthenia paralleled the febrile course. During this course a daily dose of 4,000,000 units of penicillin was chosen arbitrarily and administered intramuscularly in divided doses of 500,000 units dissolved in 2 cc. of sterile saline. Treatment was continued for 28 days. During this time repeated dose response curves were defined following individual injections of 500,000 units of penicillin. An effort was made to determine the curve at the same time of day (between 9 A.M. and 12 NOON) in order that the relationship to meals, fluid intake and diurnal changes in temperature would be comparable. Caronamide was administered for 25 days, the daily dose varying from 12 to 24 gm. The drug was administered orally at 3 hourly intervals and penicillin was injected simultaneously.

Inasmuch as a pharmacologically active compound was being administered for the purpose of temporarily inhibiting a function of the renal tubules, an effort was made to determine whether or not there was any interference with renal function as indicated by the elimination of blood urea nitrogen, creatinine and uric acid. Weekly determinations during the 30 day

TABLE 1.—SENSITIVITY TESTS ON M. C.

W 51—Rheum. Ht. Dis. and Subac. Bact. Endoc. (Strep. viridans)

Dates	Blood cultures	Penicillin			Streptomycin
		Yellow	G	X	
1946					
Aug. 1	Pos.	..	No tests		
		Course I—14,400,000 u. of Penicillin			
Oct. 26	Pos.	0.03 u./cc.			
		Course II—37,500,000 u. of Penicillin			
Dec. 31	Pos.	0.50 u./cc.			
1947					
Jan. 2	Pos.	0.50 u./cc.	..	..	0.5 u./cc.
		Course III—45 Gm. of Streptomycin			
Feb. 1	Pos.	1.00 u./cc.	1.0 u./cc.	..	5.0 u./cc.
		Course IV—24 Gm. of Streptomycin			
Feb. 10	Pos.	0.50 u./cc.	0.5 u./cc.	0.5 u./cc.	10,000 u./cc.

period of observations showed no change in the values of these substances in the blood (Chart 1).

The skin rash which appeared on the 16th day of therapy was a generalized, morbilliform rash of the type commonly seen in association with drug sensitivity. Since we were employing a new, relatively untried compound, caronamide was immediately suspected of being the cause of this rash. Our dermatologic consultants, however, regarded it as similar to those commonly encountered during penicillin therapy. It was recommended that crystalline penicillin G be substituted for ordinary amorphous (yellow) penicillin. The supply of crystalline penicillin G on hand did not permit this change. Treatment with amorphous (yellow) penicillin and caronamide was continued, and "benadryl" was administered.

Contrary to common experience, "benadryl" appeared to cause excitation and was therefore discontinued. "Pyribenzamine" was better tolerated. Our efforts to learn something concerning the renal excretion of "benadryl" were unavailing. The excitation of the patient apparently produced by the administration of "benadryl" led to the speculation that caronamide might be inhibiting the excretion of this compound.

On the 22nd day of continuous penicillin therapy, a sufficient supply of crystalline penicillin G became available and thereafter this type of penicillin was used exclusively. The skin rash faded despite continued treatment with penicillin and caronamide so that on the 28th day of treatment, the patient's skin was clear. Our reason for believing that this skin rash was due to an impurity present in the ordinary amorphous (yellow) penicillin will be referred to later. There seems to be little doubt that the secondary temperature rise reflected the appearance of drug sensitivity rather than reactivation of the bacterial endocarditis. On the 9th day of this course of treatment the blood cultures became negative and remained so.

The dose response curves indicated in Chart 1 are presented in detail in Chart 2.

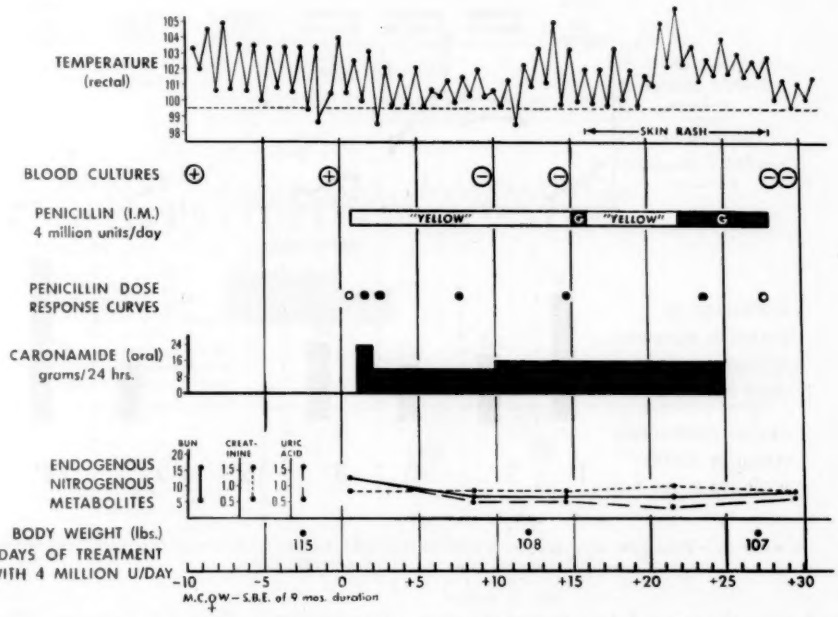

CHART 1.—Penicillin—caronamide antibiotic therapy (Course 5).

Each dose response curve was defined following an intramuscular injection of 500,000 units of penicillin. Blood was withdrawn for assays 15 minutes, 30 minutes, 1, 2 and 3 hours after individual doses of penicillin. The precaronamide control curve began at 16.54 units of penicillin per cc. of plasma at 15 minutes, and at the end of 3 hours had declined to 2.71 units of penicillin per cc. Immediately after the definition of this curve, caronamide was administered orally 3 gm. every 3 hours until the following day at which time the second dose response curve was determined. Throughout this entire 3 hour period, the penicillin plasma concentration was approximately 31 units of penicillin per cc. The dose of caronamide was reduced to 12 gm. per day or 1.5 gm. every 3 hours for 24 hours and on the 3rd day the curve showed the penicillin plasma concentrations were 62.59 units per cc. at 15 minutes and at the end of 3 hours had declined to only 20.67 units per cc. It is not entirely clear why, on 12 gm. of caronamide, the plasma concentrations of penicillin should have been higher than when 24 gm. were given. It is suggested that there was a carry-over effect from the previous day of treatment with 24 gm. of caronamide. The likelihood of this explanation is strengthened by the observation that after 12 gm. per day for 5 days, the dose response was little different from the precaronamide control nerve. Since 12 gm. per day appeared to be ineffective, the dose of caronamide was increased to 16 gm. per day. After 4 days on this schedule, a dose response curve was defined, and it was found that the plasma concentrations had increased so that 15 minutes after an injection of penicillin the concentration was 31.46 units per cc. and at the end of 3 hours was still 15 units per cc. After 9 days, however, the dose response curve again was little different from the precaronamide control curve. Inasmuch as the patient's condition had improved, and the blood cultures had been negative for several weeks, this course of therapy was terminated. Before doing so, however, a postcaronamide control curve was obtained. This second control curve begins at 20.89 units per cc. and declined during 3 hours to 1.99 units.

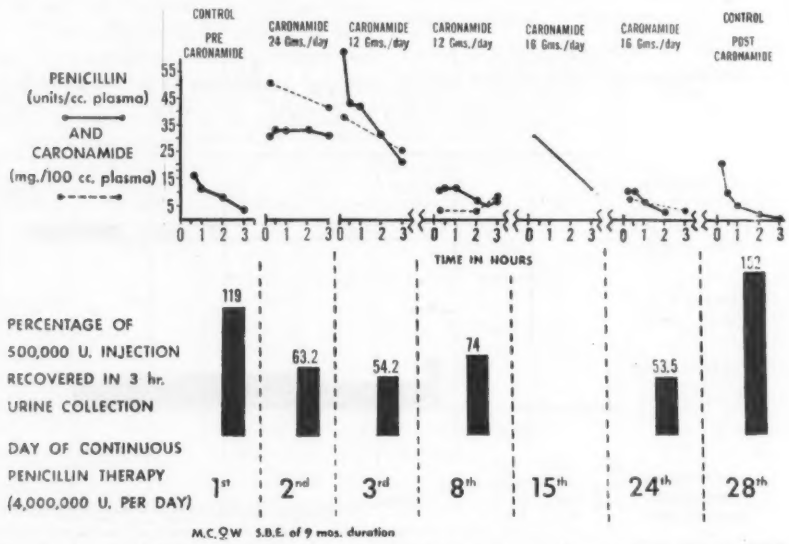

CHART 2.—Penicillin dose response curves (periods during continuous therapy) following intramuscular doses of 500,000 units.

Quantitative urinary collections were made while the above curves were described and in both of the control periods more than 100% of the injected dose of penicillin was recovered in the urine. The urinary recoveries of penicillin during therapy with caronamide averaged 61%. It might be expected that, if caronamide blocks the excretion of penicillin by the renal tubules, the elevation of plasma concentration of penicillin might be reflected in a temporarily reduced amount of penicillin in the urine. The findings

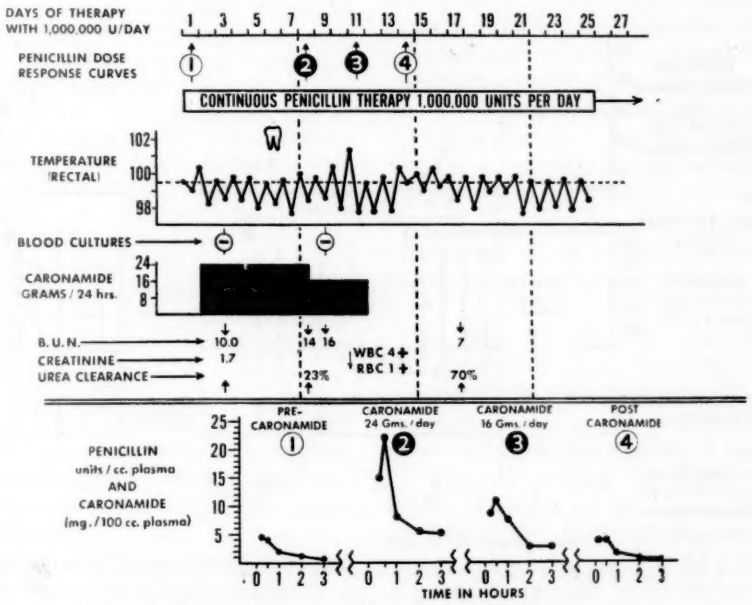

CHART 3.—Penicillin—caronamide antibiotic therapy (Course 6).

on the days when plasma levels of penicillin were increased and recoveries in the urine reduced are compatible with this expectation. On the other hand, on the 8th and 24th days (Chart 2) the recovery of penicillin in the urine was below the expected percentage, and at the same time the plasma concentrations of penicillin were not enhanced. At the present time we are unable to explain this apparent discrepancy.

Caronamide and penicillin plasma concentrations were determined simultaneously and the correlation between the concentrations of caronamide in the plasma and the enhancing effect upon the plasma concentration of penicillin is apparent.

Although the patient showed no clinical or laboratory evidence of relapse, another course of antibiotic therapy was given to insure against reactivation of a possible latent infection. The sixth course of antibiotic therapy (Chart 3) began 1 week after the termination of Course V. One million units of penicillin were given daily for 25 days and crystalline penicillin G was used throughout. Intermittent intramuscular injections of 125,000 units were given every 3 hours. Caronamide in doses of 3 gm. every 3 hours was given for a period of 8 days. Thereafter, for an additional 4 days caronamide was given in a dose of 16 gm. per day, or 2 gm. every 3 hours. Despite the concomitant administration of penicillin G and caronamide, no skin rash appeared. For this reason we are inclined to feel that the skin rash previously noted was probably due to an

impurity in the amorphous (yellow) penicillin.

Following the 8 days of caronamide therapy at the level of 24 gm. per day the blood urea nitrogen rose slightly from its previous level; the urea clearance fell to 23% and cellular elements were present in the urine. Within 10 days after discontinuance of caronamide the blood urea nitrogen returned to the previous level of 7 mg. per 100 cc., the urea clearance rose to 70% and microscopic examination of the urine showed disappearance of both red and white blood cells. The rectal temperatures during this course of antibiotic therapy were in sharp contrast to those during Course V, and this normal temperature record paralleled the patient's general improvement.

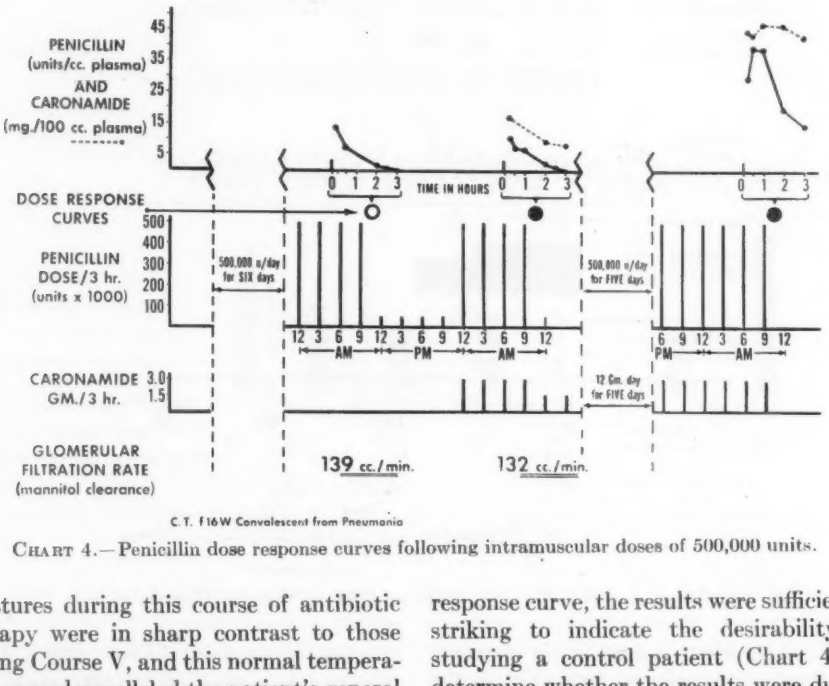

CHART 4.—Penicillin dose response curves following intramuscular doses of 500,000 units.

During Course VI both control and caronamide modified dose response curves were obtained as before. In the precaronamide control curve at 15 minutes the plasma concentration of penicillin was 4.19 units per cc. and at the end of 3 hours, only 0.25 unit per cc. When caronamide was administered in a dose of 3 gm. every 3 hours, the concentration of penicillin in the plasma was 14.77 units per cc. at 15 minutes, and at the end of 3 hours was still 5.42 units per cc. On a smaller dose of caronamide, 2 gm. every 3 hours, the plasma concentration of penicillin at the end of 15 minutes was 8.23 units per cc. and at the end of 3 hours was still 2.45 units per cc. The postcaronamide control curve compared favorably with the precaronamide curve; at 15 minutes the plasma concentration was 4.11 units per cc. and at 3 hours, 0.17 unit per cc.

Although the enhancing effect of caronamide upon the plasma concentrations of penicillin was not apparent in every dose response curve, the results were sufficiently striking to indicate the desirability of studying a control patient (Chart 4) to determine whether the results were due to a unique patient situation.

Control Case. Patient C. T., a 16 year old white girl convalescing from pneumonia, had been receiving 500,000 units of penicillin per day for 6 days prior to our observations. In order to make the dose response curves obtained from this patient comparable to those obtained from Patient M. C., the same doses of penicillin were used. Injections of 500,000 units of penicillin were given prior to obtaining the curves (Chart 4).

A control curve without caronamide showed the plasma concentration at 15 minutes to be 13.9 units per cc. and at the end of 3 hours 0.87 unit per cc. The following day the patient was given 4 intramuscular injections of penicillin 500,000 units per injection, and 4 oral doses of caronamide, 3 gm. every 3 hours. The plasma concentrations of penicillin were not increased; at 15 minutes there were 10.3 units per cc. and at 3 hours, 0.87 unit per cc. During the definition of this penicillin dose response curve, the caronamide concentrations varied between 17 and 8 mg. per 100 cc. and these concentrations did not inhibit penicillin excretion.

For 5 days this patient received 500,000 units of penicillin per day and 12 gm. of caronamide, then the effect of caronamide was reëvaluated. Six injections of penicillin at 3 hourly intervals (500,000 units per injection) and 3 gm. of caronamide were given orally every 3 hours for 6 doses. The enhancing effect upon penicillin plasma concentrations was marked; at 15 minutes the concentration was 29.95 units per cc. and at the end of 3 hours it was still 14.98 units per cc. While these values were obtained the caronamide plasma concentration varied between 45 and 43 mg. per 100 cc. and caronamide obviously inhibited tubular penicillin excretion.

Thus, in the second patient as in the first, there was good correlation between the caronamide plasma concentration and the enhancing effect upon penicillin plasma concentrations.

Discussion. Experimental work in animals has shown that caronamide is a pharmacologically active compound[2,8] of relatively low toxicity[5] which is capable of completely suppressing the excretion of penicillin by the renal tubules.[6] In mice[27] it has been shown that the intensity of penicillin therapy can be much increased by the concomitant administration of caronamide and penicillin. This has been particularly well demonstrated by the survival rate of mice infected with Type I pneumococci and typhoid bacilli of the Panama strain. This laboratory background prompted the preliminary clinical investigations with caronamide which have shown that, both in children[14] and adults[15] that penicillin plasma concentrations can be enhanced from 3 to 7 fold by the oral administration of caronamide. This enhancing effect is noted when penicillin is administered both orally and intramuscularly.

The relative resistance to penicillin of the infecting organism in the case of subacute bacterial endocarditis here described justified the use of the new compound in an effort to enhance penicillin plasma concentrations. There is an increasing awareness that it is almost impossible to maintain a "level" of penicillin in the plasma, but it has been stated that an effort should be made to exceed by 5 times the penicillin concentration which is required *in vitro* to inhibit growth of the infecting organism.[17,19] Treatment of our case in accordance with the above criterion would have required a penicillin plasma concentration of approximately 5 units. To maintain such plasma concentration of penicillin without the aid of some enhancing agent would require enormous quantities of penicillin.

The dose of 4,000,000 units per day was chosen arbitrarily but from the precaronamide and the postcaronamide control dose response curves 500,000 units given intramuscularly every 3 hours fell far short of meeting the above requirement. With the aid of caronamide it was possible to elevate the plasma concentration to remarkable heights; 30 to 60 units of penicillin per cc. Such concentrations have seldom been attained with any dose of penicillin alone and to do so in the presence of normal renal function would require at least 10,000,000 units of penicillin or more per day.

Examination of the dose response curves modified by varying caronamide doses shows that the enhancing effect of the same dose of caronamide was not constant. For example, 16 gm. per day showed enhancement of penicillin plasma concentrations on the 15th day (Chart 3) but 9 days later, on the 24th day, no effect was noted. During Course VI when 16 gm. were given (Course III, Chart 4) increased levels were

observed. In the case of Patient C. T. (Chart 4) 12 gm. given in four 3 gm. doses at 3 hourly intervals failed to enhance penicillin concentrations, whereas 18 gm. given in six 3 gm. doses showed marked effect.

The effective dose of caronamide is not uniform in terms of grams per day; enough drug must be given to insure a plasma concentration of caronamide that is adequate to inhibit tubular excretion of penicillin. Although the method used[13] in this investigation to determine caronamide in the plasma is impractical for routine use,* it has shown a good correlation between caronamide and penicillin plasma concentrations. Both of the patients were regarded as having normal renal function. With plasma concentrations of caronamide less than 10 mg. per 100 cc. Patient M. C. showed no enhancing effect on penicillin levels; similarly, concentrations of less than 20 mg. per 100 cc. in the case of Patient C. T. were ineffective. In both patients concentrations between 30 and 50 mg. per 100 cc. definitely inhibited penicillin excretion. It is suggested that plasma caronamide concentrations between 20 to 30 mg. approximates that required to inhibit excretion of penicillin by normal renal tubules.

Studies already completed indicate that the effective dose of caronamide varies from patient to patient. This is understandable since the pharmacologic action of caronamide is superimposed upon the individual patient's renal function. It is probable that all grades of renal impairment exist in subclinical form and this must be particularly true in the more advanced age groups. It may be anticipated, therefore, that an individual who has some impairment of tubular function will require less caronamide to inhibit completely the capacity of his tubules to excrete penicillin than the patient who has normal tubules. That there is a difference in caronamide requirements for the treatment of individuals of different ages has already been shown.[23] Further, it has been found that, in children, doses as large as those that have been used in adults, 24 gm. per day, may be necessary to contain the maximal enhancing effect upon penicillin plasma concentration.[14]

The individualization of caronamide dosage is desirable and will be practical when suitable methods for assaying caronamide in the plasma are developed. The method used for the determination of plasma caronamide concentrations in this investigation is impractical for routine use but other methods have already been devised.[11,28] The commonly used PSP test may be a readily available method of determining the effective dose of caronamide. Phenolsulfonphthalein (phenol red) is excreted by the same tubular excretory mechanism by which penicillin, diodrast and para-aminohippuric acid are eliminated, and it has been noted in patients under our care that the excretion of phenolsulfonphthalein is quickly inhibited by adequate dosage of caronamides. It probably can be assumed that, if sufficient caronamide is given to suppress the excretion of this dye, the excretion of penicillin is also being inhibited. Preliminary observations on this point have shown a prompt drop of the normal excretion rate of phenolsulfonphthalein to 25% and 35% under treatment with caronamide and with cessation of caronamide administration, the clearance of phenolsulfonphthalein rapidly returns to normal.

From the available information caronamide appears to have a low order of toxicity. Nausea and vomiting have been noted occasionally when excessively large doses were used, and at least 1 instance of drug fever and diffuse skin rash due to coronamide has been observed.[16] It would be completely unexpected, however, if some toxic manifestations are not observed as use of the compound becomes more widespread.

Heavy caronamide dosage may have produced a transient renal impairment as

* A 48 hour alkaline dialysate of plasma is analyzed by measuring the ultraviolet absorption at 280 m.u. in the Beckman ultraviolet spectrophotometer. This method is accurate within 10 to 20%.

indicated by cellular elements in the urine, a slight increase in the blood urea nitrogen, and a decrease in urea clearance (Chart 3). These changes occurred during a period of apparent arrest of the subacute bacterial endocarditis and cannot reasonably be attributed to the infection itself. The dental extraction which immediately preceded the onset of the urinary and renal function abnormalities could conceivably have precipitated an acute nephritis, but neither the immediate findings nor the subsequent course support this possibility. It is probable that these manifestations were, indeed, due to caronamide but the rapidity with which the urine cleared and the urea clearance returned to normal indicate the transient character of the renal irritation.

Caronamide is administered in relatively large doses and is excreted in the urine, presumably by glomerular filtration. In order to obviate the likelihood of crystalluria an adequate urinary output (1500 cc.) should be maintained. Caronamide is precipitated in an acid urine (below pH 5.5) and it may be desirable in some instances to alkalinize the urine. The maintenance of an adequate urinary output is probably better protection against renal complications than relying upon the administration of alkalinizing agents.

The rate of disappearance of penicillin from the plasma following an intramuscular dose is, in itself, a good test of renal function which reflects chiefly tubular function. Since 80% of the penicillin in the urine is excreted by the tubules, impairment of the tubules should result in delayed penicillin excretion. The close correspondence between the control curves before and after 2 courses of caronamide therapy extending over 40 days is reasonable evidence that the inhibition of the penicillin excretory mechanism was reversible.

The response of this patient to large doses of penicillin and the concomitant administration of caronamide has been good but no direct claim is made for caronamide in obtaining this result for certainly the doses of penicillin used were far in excess of those which had been tried previously. It may well be that if 4,000,000 units of penicillin alone had been given for 28 days, this patient would have responded similarly. We can say, however, that with caronamide the plasma penicillin concentrations following 1,000,000 and 4,000,000 units per day were increased over those anticipated and obtained with the same doses of penicillin alone.

Summary and Conclusion. A patient with subacute bacterial endocarditis due to a strain of *Streptococcus viridans* relatively resistant to penicillin was successfully treated with intramuscular penicillin and oral caronamide.

Even when the dosage employed is measured in millions of units per day, the plasma levels of penicillin can be elevated by the administration of caronamide. The effect of caronamide in the treatment of this patient was equivalent to the injection of additional millions of units of penicillin.

REFERENCES

1. Avery, N. L., Jr., Mayer, O. B., and Nelson, R. C.: Ann. Int. Med., **24**, 900, 1946.
2. Beyer, K. H.: Science, **105**, 94, 1947.
3. Beyer, K. H., Flippin, H., Verwey, W. F., and Woodward, R.: J. Am. Med. Assn., **126**, 1007, 1944.
4. Beyer, K. H., Mattis, P. A., Patch, E. A., and Russo, H. F.: J. Pharm. and Exp. Ther., **84**, 136, 1945.
5. Beyer, K. H., McKinney, S. E., Tillson, E. A., and Green, C. W.: To be published.
6. Beyer, K. H., Miller, A. K., Russo, H. F., Patch, E. A., and Verwey, W. F.: Am. J. Physiol. (in press).
7. Beyer, K. H., Peters, L., Woodward, R., and Verwey, W. F.: J. Pharm. and Exp. Ther., **82**, 310, 1944.
8. Beyer, K. H., Russo, H. F., Patch, E. A., and Tillson, E. A.: To be published.
9. Beyer, K. H., Verwey, W. F., Woodward, R., Peters, L., and Mattis, P. A.: Am. J. Med. Sci., **209**, 608, 1945.

10. Beyer, K. H., Woodward, R., Peters, L., Verwey, W. F., and Mattis, P. A.: Science, **100**, 107, 1944.
11. Brodie, B. B., and Earle, D. P.: Unpublished data.
12. Bronfenbrenner, J., and Favour, C. B.: Science, **101**, 673, 1945.
13. Ciminera, J. L.: Unpublished data.
14. Corneal, B., Kildrick-Smith, G., and Scott, T. S. McN.: Personal communication.
15. Crosson, J. W., Boger, W. P., Shaw, C. C., and Miller, A. K.: J. Am. Med. Assn. (in press).
16. Duncan, G. G.: Personal communication.
17. Favour, C. B., Janeway, C. A., Gibson, J. G., 2nd.,and Levine, S. A.: New England J. Med., **234**, 71, 1946.
18. Loewe, L., Rosenblatt, P., and Alture-Werber, E.: Am. Heart J., **32**, 327, 1946.
19. Loewe, L.: Bull. New York Acad. Med., **21**, 59, 1945.
20. Mokotoff, R., Brams, W., Katz, L. N., and Howell, K. M.: Am. J. Med. Sci., **211**, 395, 1946.
21. Rake, G., and Richardson, A. P.: Ann. New York Acad. Sci., **48**, 143, 1946.
22. Rammelkamp, C. H., and Bradley, S. W.: Proc. Soc. Exp. Biol. and Med., **53**, 30, 1943.
23. Seeler, A. O., Wilcox, C., and Finland, M.: J. Lab. and Clin. Med. (in press).
24. Smith, A. L., Jr.: Personal communication.
25. Spaulding, E. H., Bondi, A., Jr., and Early, E.: Science, **105**, 210, 1947.
26. Sprague, J. M., Ziegler, C., Cragoe, E. J., and Miller, C. S.: To be published.
27. Verwey, W. F., and Miller, A. K.: To be published.
28. Ziegler, C., and Sprague, J. M.: Unpublished data.

CONVULSIVE STATE IN DIABETES

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In the relatively small but important group of cases of labile diabetes, the frequency of episodes of hypoglycemia and the difficulty of satisfactory control of the glycosuria are well recognized. This group, which is composed almost entirely of younger patients, has always been a severe problem not only to the general practitioner but also to the specialist in diabetes.

The factors that underlie the unpredictability of sudden changes from glycosuria and ketonuria to convulsive states have never been clearly delineated. We have observed 2 patients who tend to shed some light on this situation. Both are young women with "brittle" diabetes, and both had experienced repeated, severe "insulin shocks." One of them died in the hospital; the other is still under observation. The case histories follow:

Case Reports. Case 1. M. B., a white female, was first admitted to The Mount Sinai Hospital in August 1933, at the age of 11, for regulation of her diabetes. The onset of the diabetes had occurred at the age of 6 with polyphagia, polyuria, polydipsia and loss of weight. In the intervening 5 years she had entered other hospitals on 3 occasions because of frequent hypoglycemic episodes, most of which were described as severe. The patient had been raised in a foster home and had never been instructed in the significance of the disease nor in estimating her diet. It was noted that she was not gaining weight and that marked glycosuria was present.

Family history revealed the presence of diabetes in an aunt. Past history was noncontributory; physical examination and laboratory findings other than hyperglycemia and glycosuria were normal. During her stay in the hospital she was given a diet of carbohydrate (85 gm.), protein (65 gm.), fat (100 gm.), with regular insulin (25–8–25 units) before each of the 3 meals, respectively. Although she was not kept sugar-free on this regimen, and in fact had occasional acetonuria, she still had occasional severe insulin reactions, one of which was accompanied by a generalized convulsion.

Second admission: October 1934. One day after discharge from the hospital she had a severe shock accompanied by convulsions.

Third admission: July 1935. She was admitted for tonsillectomy and adenoidectomy because of repeated sore throats. In the interim she had been having occasional insulin reactions. The operation was uncomplicated.

Fourth admission: June 1936. This was for a traumatic slipped epiphysis of the lower end of the right radius and a fracture of the right ulnar styloid. Healing was satisfactory.

Fifth admission: October 1936. This admission was due to pain in the ear, sore throat and fever. She was disoriented, slightly confused and in obvious diabetic ketosis. The ketosis responded promptly to treatment. Examination revealed a palpable liver edge, normal ocular fundi, and no focal neurologic signs. The patient stated that she had been having frequent, severe nocturnal insulin shocks, as well as occasional dizzy spells. It was now suspected that the picture was complicated by some factor in addition to insulin hypoglycemia. However, in the 2 instances during this admission when she was observed to be unconscious with convulsive movements, she responded to the administration of 50% glucose intravenously. The severity of the diabetes continued to fluctuate; that is, she would pass rapidly from marked glycosuria, even with acetonuria, to severe hypoglycemic

reaction. When discharged from the hospital she was taking 60 units of protamine zinc insulin daily.

Sixth admission: April 1938. The patient was again admitted for frequent nocturnal "shocks," although she was having daily glycosuria. Examination was essentially negative. The blood pressure was 105/75. Laboratory examinations were within normal limits. A roentgenogram of the chest was normal; a roentgenogram of the skull did not show any evidence of increased intracranial pressure and the sella turcica was normal. An electrocardiogram revealed changes in the T waves indicating some involvement of the ventricular musculature.

Further observation in the hospital revealed that there were frequent seizures. Occasionally these were attended by clonic and tonic movements; champing movements of the jaws, rolling of the eyeballs, and biting of the tongue. The attacks were followed by headache. They would cease spontaneously; complete amnesia occurred afterward. The attacks tended to increase in frequency at the onset of menstrual periods. Occasionally the blood sugar was elevated up to 190 mg. per 100 cc. during the episode. A moderate regression was induced by the use of phenobarbital. It was therefore inferred that in addition to hypoglycemic episodes, she was suffering from another factor, probably of the nature of an idiopathic epileptiform state.

Seventh and final admission: September 1938. She was admitted in severe ketosis which followed protracted vomiting and neglect in the taking of insulin. She was drowsy, irrational, markedly dehydrated; the blood pressure was down to 76/60. She was treated vigorously and brought out of the ketotic state. She again displayed convulsive manifestations, which most often occurred at night. The blood sugar level varied in different attacks; at times it was normal or elevated, whereas at other times it was low. One nocturnal seizure was actually observed and was described as follows: it was ushered in by an inspiratory crow; the head and eyes deviated to the right, the left arm was extended and the right arm was flexed at the side; there then ensued coarse convulsive tremors of the shoulders and extremities, frothing at the mouth, stertorous respirations and cyanosis; the pupils were dilated and fixed to light; a Babinski sign was present on the right. Dilantin was started, with no obvious effect.

Several days later, the patient was found dead in bed. Presumably she had died in her sleep, and without any outcry or activity to attract the attention of either the nurses or the patients on the ward. Postmortem blood sugar was 330 mg. per 100 cc., which ruled out hypoglycemia as a cause of death.

Postmortem Examination. The body was that of a well-nourished white female, aged 18. There was good panniculus. The heart was normal. The aorta and the larger coronary branches showed scattered, flat, atheromatous plaques with no narrowing of the lumens. The liver weighed 1600 gm., was smooth and showed normal lobular markings. The lungs, gall bladder, adrenal glands, kidneys, uterus and ovaries were grossly normal. The gastro-intestinal tract showed prominence of the lymphoid tissue in the ileum and large intestine. The thymus weighed 35 gm. Microscopically there was congestion of the lungs, liver, spleen and kidneys. The hepatic cells were filled with glycogen. The pancreatic islets appeared normal. There was hyperplasia of the mesenteric nodes. Brain: grossly, the meninges were smooth and shining; the meningeal blood-vessels were moderately engorged. The pituitary was grossly normal. There was prominence of the blood-vessels most marked in the frontal and parietal region, and also present in the pons, but absent in the medulla.

Sections of the frontal lobe, stained with hematoxylin-eosin, disclosed several arachnoid cell clusters in the leptomeninges. The nerve cells of the cortex presented a diversity of degenerative changes. Some were markedly swollen; many, however, were shrunken and contained pyknotic nuclei, while some contained finely granular, deeply staining oval nuclei with an occasional enlarged nucleolus. There were other cells in which it was difficult to distinguish nucleus from cytoplasm. The outline of some cells was irregular and occasionally the cytoplasm was poorly defined. Satellitosis was increased. With Nissl stains, marked central chromatolysis was seen in some areas. The Nissl substance itself was usually finely granular, diffusely scattered through the cytoplasm or concentrated at the periphery at the base of the cell. Some cells stained very faintly. Both cortex and subcortex showed marked

and diffuse gliosis. The blood-vessels were engorged. Similar though less marked changes in the nerve cells were seen in the tegmentum, pons and medulla.

Pathologic diagnosis: degenerative encephalopathy; toxic (?) encephalopathy.

Case 2. S. K., a white female, was first seen in the Out-Patient Clinic of The Mount Sinai Hospital in 1940, at the age of 13. She was treated for a keloid of the right hand; there were no other complaints or abnormal findings. In May 1943, she was admitted to another hospital in diabetic ketosis which followed 2 weeks of loss of weight, polyuria, polydipsia and pruritus, without preceding incident. She was discharged on a diet of carbohydrate (200 gm.), protein (80 gm.), fat (80 gm.) and 75 units of protamine zinc insulin each morning. Thereafter she attended our clinic. She had repeated insulin reactions and frequent glycosuria. At first her daily requirements for insulin rose to 130 units of protamine zinc insulin, but this was gradually reduced to 70 units. She spent part of the summer of 1943 in a diabetic camp; and here, too, the the frequency of insulin shocks was clearly noted.

In February 1944, she was admitted to The Mount Sinai Hospital for diabetic ketosis, and in December 1944 she was admitted for appendectomy. From then until February 1946 she had repeated admissions to several hospitals for either ketosis or shock. Blood pressure, blood cholesterol, blood proteins, chest roentgenograms, neurologic examinations and the eyegrounds were always within normal limits. Occasionally, her urine would show a trace of albumin.

In February 1946, at the age of 19, she was again admitted to this hospital in severe ketosis, following an upper respiratory infection. The ketosis responded well to energetic treatment. However, the diabetes was never too well controlled on a high carbohydrate diet, with the insulin dosage varying from 40 units of protamine zinc insulin to a 2:1 mixture of 60 units of regular insulin and 30 units of protamine zinc insulin. During this hospital stay, she continued to have many attacks of shock which were considered to be related to hypoglycemia. However, many of these were found to occur with a normal of even markedly elevated blood sugar. The seizures were noted to consist of fogging of consciousness, sucking movements of the mouth and tongue, occasionally preceded by a sensation of thirst, and often followed by a variable period of drowsiness. Convulsive movements, tongue-biting, incontinence and frothing at the mouth were constantly absent. There was complete amnesia for these episodes. Neurologic investigation revealed: normal neurologic examination, normal lumbar puncture findings and a normal skull roentgenogram. An electroencephalogram showed alpha waves with frequencies as low as 7 per second, with rare bursts of 3 and 6 per second activity. Hypoglycemic effects on the EEG were ruled out by demonstrating the persistence of these changes even in the presence of marked hyperglycemia.

Dilantin, phenobarbital and tridione were each tried in full therapeutic dosage with no measurable beneficial effect on the frequency or severity of the attacks. She was given a free diet and permitted to have glycosuria, but there was no change in her condition. Because of the fear of possible attacks, she is unable to work. At the present time she is still on a free diet with a mixture of 42 units of regular insulin and 17 units of protamine zinc insulin every morning. The seizures continue at the rate of approximately 1 every 3 days.

An electroencephalogram made in April 1947 again showed alpha waves with frequencies as low as 7.5 per second with rare bursts of 6 per second activity and some single 3 per second waves. Hyperventilation did not increase the amount of slow activity. A record taken 10 minutes after the intravenous injection of 50 cc. of 50% glucose showed the same characteristics.

Discussion. It is quite evident that definite abnormalities existed in both cases as demonstrated by the pathologic findings in the one and the electroencephalographic changes in the second. The pathogenesis of these abnormal findings and their relation to the clinical picture is a matter of great importance. Several possibilities are suggested: (1) they are the manifestations of irreversible cerebral changes following repeated insulin hypoglycemia; (2) the changes are independent of the diabetes and are actually an influence in the causation of repeated shocks;

(3) they are evidences of coincidental epilepsy.

That repeated hypoglycemic shocks can and do result in irreversible changes in both humans and experimental animals has been amply verified.[1,4,5,6] The actual pathology, while not specific, is sufficiently characteristic to be recognized when the clinical history is known. The changes consist mainly of multiple petechial hemorrhages, proliferation of the glial tissue and widely disseminated injury to the nerve cells. Similar changes are found in cerebral anoxia. Clinically, the relation of hypoglycemia to neurologic symptoms is equally apparent, so much so that it has been aptly likened to an abnormal physiologic decortication. Almost all patients with hypoglycemia have neurologic or psychiatric symptoms of one type or another. Little wonder, then, that permanent changes result, under such conditions, in an organ as sensitive as the brain.

The possibility that the cerebral changes are independent of the diabetes and insulin hypoglycemia, and are important in actually instigating frequent reactions has been suggested by Greenblatt *et al.*[2] These authors recorded abnormal electroencephalograms in 51% of their "problem diabetics" and concluded that the central nervous system plays a leading rôle in the instability. However, they did not rule out the possibility of hypoglycemic changes. Further, such a high incidence of epileptiform tendencies in diabetics is not borne out by clinical experience or available statistics.[3]

Coincidental idiopathic epilepsy must be considered in the differential diagnosis. However, aside from the atypical features of the episodes, the absence of a family history, the lack of response to fairly specific drugs, and the presence of definite pathologic changes in the brain of Case 1 make this highly untenable.

At the present time, we are further investigating the development of the syndrome in our diabetics, as well as the concomitant effect on the personality and associated visceral changes.

The importance of these observations is twofold: (1) The absolute necessity for the avoidance of hypoglycemic episodes is paramount not only for the fright of the immediate episode, but particularly for the prevention of the cumulative irreversible effects of repeated episodes. (2) In the management and treatment of these cases, one must be aware of the possibility of symptoms simulating shock resulting from causes other than hypoglycemia, so that proper treatment may be instituted.

Summary. 1. Two cases of labile diabetes associated with non-hypoglycemic reactions are presented.

2. The pathologic and electroencephalographic evidence of cerebral damage were present in these cases.

3. The most tenable assumption is that repeated severe insulin shocks produce irreversible cerebral damage.

REFERENCES

1. Baker, A. B., and Lufkin, N. H.: Cerebral Lesions in Hypoglycemia, Arch. Path., **23**, 190, 1937.
2. Greenblatt, M., Murray, J., and Root, H. F.: Electroencephalographic Studies in Diabetes Mellitus, New England J. Med., **234**, 119, 1946.
3. Joslin, E. P., Root, H. F., White, P., and Marble, A.: The Treatment of Diabetes Mellitus, 7th ed., Phila., Lea & Febiger, p. 498, 1940.
4. Malamud, N., and Grosh, L. C., Jr.: Hyperinsulinism and Cerebral Changes, Arch. Int. Med., **61**, 579, 1938.
5. Rigdon, R. H., and Fletcher, D. E.: Blindness in Ducks Accompanying Hypoglycemia, Am. J. Path., **23**, 227, 1947.
6. Weil, A., Liebert, E., aad Heilbrunn, G.: Histopathologic Changes in the Brain in Experimental Hyperinsulinism, Arch. Neurol. and Psych., **39**, 467, 1938.

THE URINARY EXCRETION OF AMINO ACIDS BY A CYSTINURIC SUBJECT*

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Cystinuria is a rare metabolic disease in which cystine stones are deposited in the kidney, cystine crystals may be found in the cornea, conjunctiva, bone marrow and other tissues and as much as 0.5 gm. of cystine[18] may be excreted daily in the urine. Looney *et al.*[16] concluded in 1923 that "The excretion of cystine (*in cystinuria*) is not simply an index of a general disturbance in the metabolism of amino acids but a definite entity confined to cystine," but the observations of several other workers are opposed to this view. Udranszky and Baumann,[19] and other investigators[18] found cadaverine and putrescine in the urine of some cystinuric subjects, while Loewy and Neuberg[15] reported that the excretion of these diamines by cystinuric patients was increased after the ingestion of arginine and lysine. Later workers[4,5,6,11,14] have shown that cystine excretion in cystinuria was increased after the ingestion of methionine, cysteine (but not cystine), glycine, alanine and glutamic acid. It has been reported[2,9,18,20,21] that aspartic acid, leucine and tyrosine were tolerated by cystinuric patients or appeared almost quantitatively in the urine.

Cystine, leucine, tyrosine, lysine and the benzoyl derivative of a substance thought to be derived from tryptophane[1,9,12] have been detected in cystinuric urines, but quantitative data are lacking. For this reason, we determined quantitatively a series of amino acids in the urine of a cystinuric subject with the aid of microbiologic methods.

Case History. R. B., a 5 year old white girl, was first admitted at the age of 2 years to the Urological Service of the Hospital of the University of Pennsylvania in 1942. At that time she ran an irregular fever, had pain on urination and voided a cloudy urine. A diagnosis of undulant fever was made. After several months she was discharged. In 1943 she was readmitted to the hospital in coma with abdominal distention and high fever. She recovered from the acute episode, but the pain on urination and the cloudy urine persisted. Roentgen rays taken at this time indicated bilateral urinary calculi. A low fat diet was advised.

The complaints continued and on Aug. 16, 1945, she was readmitted for further study. Her past medical history was non-contributory. She was a full-term baby normally delivered. Her diet was adequate. Development was normal until the stated symp-

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toms began at 20 months. The family history was negative except for 1 aunt with renal disease presumably renal calculi. Physical examination revealed a coöperative pleasant girl who appeared small for her age. Blood pressure was 100/60. Tonsils were atrophied. Remainder of the examination was normal. Laboratory studies were as follows: hemoglobin, 68%; leukocytes, 6500 per c.mm.; urine, acid with 4 to 5 white blood cells per high power field; and urine culture, *Aerobacter aerogenes* and non-hemolytic streptococci. Roentgen ray and intravenous urography confirmed the presence of a right renal calculus and a left ureteral calculus. There was a bilateral hydronephrosis. Cystoscopy under avertin anesthesia revealed a normal bladder cavity. Ureteral catheterization and pyelography confirmed these observations.

A left uretero-lithotomy was performed on Aug. 21, 1945, and the calculus was removed from the dilated ureter. Convalescence was uneventful and a right pyelolithotomy was performed on September 6. The elliptical-shaped calculus from the left ureter measured 2.4 x 0.7 cm. and the roughly triangular calculus from the right kidney pelvis measured 2 x 1.9 cm. The yellow-brown stones had finely granular surfaces and a "maple-sugar" appearance. The calculi were identified as cystine by the Sullivan test. Cystine crystals were found on several occasions. She was discharged on September 23.

She was readmitted on Oct. 22, 1945. The physical examination was unchanged except for well-healed operative wounds. She was placed on a strict diet of 1500 calories daily consisting of proteins (31 to 50 gm.), carbohydrates (119 to 220 gm.), fat (73 to 110 gm.), taken as milk, fruit juices, oatmeal, eggs, graham crackers and butter. Fluids were forced to 2000 cc. daily. On 3 consecutive days 24 hour urine specimens were collected for analysis. She was then placed on methionine, 0.3 gm., 3 times daily for 3 days, in addition to the diet. Urine samples were obtained as before.

Further studies at this time revealed: hemoglobin, 82%; leukocytes, 7200 per c.mm.; urine, acid with many white blood cells per high power field and a trace of albumin, sugar negative; blood urea nitrogen, 10 mg. per 100 ml.; fasting blood sugar, 82 mg. per 100 ml.; carbon dioxide combining power, 49 volumes per 100 ml.; uric acid, 2.2 mg. per 100 ml.; protein, 6.6 gm. per 100 ml.; chlorides, 106.6 m.eq. per liter; serum calcium, 10.3 mg. per 100 ml.; serum phosphorus, 3.9 mg. per 100 ml.; and alkaline phosphatase, 13.1 Bodansky units. The phenolsulfonphthalein excretion was 62% in 2 hours. Roentgen rays of the long bones showed no evidence of rickets and were considered normal. Intravenous urography demonstrated a residual hydronephrosis with no evidence of calculi.

Upon completion of these studies the child was discharged in good condition. She has remained symptom free and has developed normally.

Preparation of Urine Samples. Aliquots of the cystinuric urines were frozen, dried and preserved in this state, the dry material was weighed and dissolved in 0.2 N hydrochloric acid, and the acid solutions were diluted to the original volumes of the aliquots. These solutions and aliquots of the normal urine samples were deproteinized by adjusting the solutions to pH 6.8 and boiling them for 10 minutes. A few drops of acetic acid were added to dissolve phosphates and the mixtures were cooled and filtered. Aliquots taken for amino acid assay were adjusted to pH 6.8 and diluted to the desired volumes. That this treatment was without significant effect on the amino acids in the urines was indicated by assays which gave values for the boiled samples averaging 94 (90 to 100)% of those found for glutamic acid, leucine and valine in the unboiled samples.

Acid hydrolysates of the deprotenized urines were prepared by refluxing 50 ml. aliquots for 6 hours at 120° with 6 N hydrochloric acid. The amino acid values were not altered significantly by refluxing the samples for 20 hours under the same conditions. Excess hydrochloric acid was removed by distilling each solution to dryness *in vacuo* at 70 to 80°, adding 30 ml. of distilled water to the residual material and redistilling the solution to dryness *in vacuo*. Each residue was dissolved as completely as possible in hot water, the suspension was filtered, the precipitate was washed thoroughly with hot N hydrochloric acid and hot water, and the combined filtrates were diluted to 100 ml. An aliquot of each approximately 0.1 N hydrochloric acid solution was neutralized to pH 6.8, compensated by

the method described previously in this series of papers for the salt estimated to be present in the original urine and that formed by neutralization of the hydrochloric acid, and diluted to the desired volume.

The urine samples, diluted 1:1.5 (see Table 1), were treated with urease to remove urea, a possible interfering substance at this low dilution of urine. The urease was purified essentially by the method of Archibald and Hamilton[3] to remove canavanine and any other free amino acids. A 65 ml. aliquot of urine was adjusted to pH 7, an aliquot of the purified urease solution equivalent to 0.35 gm. of urease was added, and the mixture was incubated at 35 to 50° for 30 minutes. The mixture was boiled immediately for 5 minutes to inactivate the urease and any other enzymes present, 1 drop of thymolblue indicator. The organisms, the approximate dilutions of the urine samples and the incubation times are given in Table 1.

EXPERIMENTAL RESULTS. The daily excretion of 15 amino acids by 7 normal female subjects and by the female cystinuric patient over a period of 5 days is shown in Tables 2 and 3. That these data are of reasonable accuracy is indicated by the mean deviations from the mean of the values at the different levels of samples which averaged 6.4 (3.3 to 10) % for the unhydrolyzed urines and 5 (2.8 to 7.4) % for the hydrolyzed urines. Higher values up to 43% were found for aspartic acid, isoleucine, leucine and threonine in un-

TABLE 1.—ORGANISMS, URINE DILUTIONS AND INCUBATION TIMES EMPLOYED IN AMINO ACID ASSAYS

Amino acid	Urine dilutions		Organism	Incubation time (days)
	Unhydrolyzed	Hydrolyzed		
Arginine	1:2.5	1:5	*Lactobacillus casei*	3
Aspartic acid	1:2.5	1:20	*Leuconostoc mesenteroides* P–60	3
Cystine	1:40	1:40	*Leuconostoc mesenteroides* P–60	5
Glutamic acid	1:2.5	1:40	*Lactobacillus arabinosus* 17–5	3
Glycine	1:20	1:20	*Leuconostoc mesenteroides* P–60	3
Histidine	1:40	1:40	*Leuconostoc mesenteroides* P–60	5
Isoleucine	1:1.5	1:2.5	*Lactobacillus arabinosus* 17–5	3
Leucine	1:2.5	1:2.5	*Lactobacillus arabinosus* 17–5	3
Lysine	1:1.5	1:2.5	*Leuconostoc mesenteroides* P–60	5
Methionine	1:1.5	1:5	*Lactobacillus fermenti* 36	2
Phenylalanine	1:5	1:5	*Leuconostoc mesenteroides* P–60	5
Threonine	1:1.5	1:2.5	*Lactobacillus fermenti* 36	2
Tryptophane	1:20	..	*Lactobacillus arabinosus* 17–5	3
Tyrosine	1:5	1:5	*Lactobacillus casei*	3
Valine	1:2.5	1:2.5	*Lactobacillus arabinosus* 17–5	3

3 N sodium hydroxide was added and the alkaline solution was boiled for 5 minutes to remove ammonia. The hot solution was acidified with acetic acid to dissolve phosphates and to precipitate any proteins present in the urine and the urease. The suspension was cooled and filtered, the precipitate was washed thoroughly with hot water, the filtrate was adjusted to pH 6.8 and the final solution was diluted to 100 ml.

MICROBIOLOGIC ASSAY PROCEDURE. The amino acids were determined by the microbiologic procedures and techniques described by Dunn *et al.* in earlier papers of this series except that aspartic acid was determined by the method of Hac and Snell.[10] The final volumes of solutions employed in the assays were 3 ml. per 13 x 100 mm. test tube. Acid production was measured by titration with standard base using bromo-

hydrolyzed urines due primarily to the low concentration of these amino acids.

The possibility that any putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane) present in the cystinuric urine might be measured microbiologically as arginine and lysine, respectively, was investigated. Putrescine was obtained from the Eastman Kodak Company and cadaverine was synthesized as the dihydrochloride from trimethylene bromide by reduction with sodium of the intermediate trimethylene cyanide essentially by the method of Ladenburg.[13] The cadaverine was only about 93% pure since the melting point was 239° to 241° (usual accepted value, 242° to 243°[17]) and the nitrogen content determined by semi-micro Kjeldahl

TABLE 2.—AMINO ACIDS IN NORMAL FEMALE URINES (MG. PER HOURS)*

	Subject 1		2		3		4		5		6		Average		7	
Amino acid	Unhyd.	Hyd.	Unhyd.	Hyd.	Unhyd.	Hyd.	Unhyd.	Hyd.	Unhyd.	Hyd.	Unhyd.	Hyd.	Unhyd.	Hyd.	Unhyd.	Hyd
Arginine	10.6	15.5	7.0	14.1	9.4	14.8	12.8	22.4	9.9	16.0	14.0	15.5	16.0	16.4	4.7	12.1
Aspartic acid	2.0	51.0	3.8	50.8	3.5	49.8	5.1	95.0	1.7	60.8	9.2	59.0	4.2	61.1	0	18.0
Cystine†	64.0	48.0	74.0	48.0	86.0	76.0	106.0	102.0	106.0	106.0	100.0	93.0	89.3	78.9	100.0	38.5
Glutamic acid	11.6	242.0	Tr.	220.0	8.9	256.0	7.9	190.0	6.6	328.0	8.7	235.0	7.3	244.0	0	128.0
Glycine‡	335.0	305.0	1340.0	740.0	241.0	325.0	590.0	560.0	1030.0	660.0	605.0	602.0	690.0	532.0	145.0	91.0
Histidine	118.0	121.0	26.0	40.0	91.0	89.0	99.0	102.0	81.0	99.0	71.0	83.0	81.0	89.0	51.4	77.0
Isoleucine	4.0	11.4	2.0	10.1	2.7	15.9	6.0	18.5	5.8	16.4	6.9	15.3	4.6	14.6	0	2.1
Leucine	3.4	10.2	1.8	9.4	3.6	10.1	6.2	18.3	2.7	12.4	3.4	11.6	3.5	12.0	4.9	9.6
Lysine	16.6	66.0	6.1	43.0	27.2	68.0	14.0	72.0	15.9	55.0	22.6	63.0	17.1	62.8	5.8	13.6
Methionine	0.7	6.6	1.0	9.6	0.9	8.6	2.6	15.4	1.1	11.8	4.5	8.1	1.8	10.0	1.5	38.4
Phenylalanine	6.1	5.9	2.5	5.7	3.4	9.2	10.6	17.0	10.6	10.6	5.5	7.1	7.8	29.9	6.2	27.5
Threonine	8.0	26.6	2.8	23.6	9.5	29.2	10.0	36.0	11.6	34.2	4.8	29.7	7.8	§	6.7	§
Tryptophane	8.4	§	4.2	§	7.1	§	11.0	§	10.7	§	5.6	§	10.6	20.5	5.7	12.5
Tyrosine†	11.5	20.5	6.8	16.5	10.9	21.9	13.2	25.5	13.3	21.3	8.0	17.2	2.8	14.6	0	8.2
Valine	3.3	12:1	Tr.	12.1	3.3	15.1	2.7	19.0	4.0	16.7	3.3	12.8				
Total	603.0	942.0	1478.0	1243.0	508.0	989.0	897.0	1293.0	1305.0	1448.0	873.0	1252.0	951.0	1195.0	337.0	485.0

* All of the urines were neutral to litmus except that of Subject 7 which was acid (pH 5.9). The urine of Subject 6 contained a trace of protein; all other urines contained no protein. The age, weight in pounds, height in inches, total ml. volume of urine and grams of total nitrogen in the 24 hour urine, respectively, of the subjects indicated by the numbers in the parentheses were: (1) 18, 125, 64, 660 and 7.2; (2) 19, 135, 62.5, 1122 and 9.1; (3) 23, 110, 58, 1270 and 7.8; (4) 33, 120, 63, 1040 and 8.9; (5) 21, 114, 64, 1110 and 9.5; (6) 32, 92, 60, 1650 and 7.7; and (7) 8, 40, 47, 570 and 7.7.

† Values for hydrolyzed samples minimum since cystine and tyrosine are known to be partially destroyed by treatment with acid in the presence of carbohydrates and other oxygenated substances.

‡ It is considered probable that the lower values found for glycine in the acid-hydrolyzed urines may be accounted for by hydrolysis of the hippuric acid and by the higher activity (per mole) of hippuric acid than glycine for *L. mesenteroides* P-60 (unpublished data obtained by Ruth Malin in the laboratory of one of us (M. S. D.).

§ Not determined since tryptophane is destroyed by treatment with acid.

TABLE 3.—AMINO ACIDS IN CYSTINURIC FEMALE URINES (MG. PER 24 HOURS)*

	Urine sample No. 1072		1073		1074		1075		1076†		Average	
Amino acid	Unhyd.	Hyd.	Unhyd.	Hyd.	Unhyd.	Hyd.	Unhyd.	Hyd.	Unhyd.	Hyd.	Unhyd.	Hyd.
Arginine	73.0	34.6	112.0	112.0	224.0	265.0	124.0	140.0	130.0	136.0	127.0	137.0
Aspartic acid	1.3	‡	5.4	32.2	4.6	64.0	1.2	34.0	2.0	27.8	2.9	39.5
Cystine§	123.0	‡	210.0	224.0	567.0	470.0	289.0	206.0	266.0	149.0	291.0	262.0
Glutamic acid	2.0	‡	28.8	125.0	12.1	230.0	2.5	81.0	8.8	‡	10.8	145.0
Glycine	70.0	84.0	150.0	114.0	369.0	304.0	123.0	92.0	409.0	204.0	224.0	160.0
Histidine	14.0	51.5	22.3	18.7	49.0	56.0	27.6	30.0	19.3	22.1	26.4	37.7
Isoleucine	1.2	0.9	3.1	9.4	2.1	20.6	1.8	13.2	2.0	7.4	2.0	10.3
Leucine	1.2	0.6	3.0	12.9	2.7	38.4	1.3	21.1	1.0	10.7	1.8	16.7
Lysine	78.0	136.0	164.0	198.0	496.0	500.0	238.0	234.0	184.0	200.0	232.0	253.0
Methionine	3.0	‡	9.3	10.3	25.5	54.6	6.2	11.3	11.2	16.0	11.0	23.0
Phenylalanine	1.5	15.5	3.4	13.6	10.2	32.2	2.3	16.2	2.4	8.2	3.8	17.5
Threonine	5.1	‡	9.9	15.3	17.8	30.8	16.2	23.9	12.4	16.0	12.3	21.5
Tryptophane	1.5	¶	2.9	¶	5.6	¶	2.6	¶	2.1	¶	2.9	¶
Tyrosine§	3.1	1.0	7.7	11.8	13.1	30.4	6.7	16.6	5.8	13.6	7.3	14.7
Valine	0.9	1.3	3.4	10.5	1.1	25.1	1.0	16.6	0.7	8.9	1.4	12.5
Total	379.0	325.0	735.0	908.0	1800.0	2121.0	843.0	936.0	1057.0	820.0	957.0	1150.0

* The total ml. volume and grams of total nitrogen, respectively, for the urine samples (numbers given in the parentheses) were: (1072) 550 and 4, (1073) 750 and 8.8, (1074) 895 and 24.8, (1075) 975 and 8.1, and (1076) 475 and 5. The subject ingested 0.3 gm. of dl-methionine 3 times during the day prior to the collection of Sample 1073 and daily on the days during which Samples 1073 and 1074 were collected.

† 12 hour sample.

‡ Not determined because of insufficient sample.

§ Values for hydrolyzed samples minimum since cystine and tyrosine are known to be partially destroyed by treatment with acid in the presence of carbohydrates and other oxygenated substances.

¶ Not determined since tryptophane is destroyed by treatment wih acid.

was 14.9% (theory, 16%) for the dihydrochloride. It was found that putrescine did not support the growth of *L. casei* on an arginine-deficient medium and that it did not inhibit the growth of this organism on the same medium supplemented with an adequate amount of arginine. Entirely analogous results were obtained with cadaverine.

Discussion. It would appear from the data given in Tables 2 and 3 that arginine, cystine, histidine and phenylalanine in normal female urine and arginine, cystine and lysine in the cystinuric urine were present largely in the unconjugated state since essentially the same values for these amino acids were found before and after hydrolysis of the urines. It may be possible, but is not probable, that conjugated forms of these amino acids with nearly identical activity for the assay microorganisms were present. Since the total amino acids determined after hydrolysis averaged about 45% higher for the normal urines and about 20% higher for the cystinuric urines, it may be concluded that large proportions of amino acids other than those stated existed in conjugated forms. This increase was especially marked in the case of glutamic acid. These observations coincide generally with those reported by Dunn *et al.*[7] for normal male urines.

In our 7 normal subjects the amino acid excretion shows a remarkably uniform pattern, regardless of age differences (age varying from 8 to 33 years).

Marked changes from the normal occurred in our case of cystinuria in the excretion of some amino acids both in absolute and relative amounts. The most significant increases in the unhydrolyzed urines were found for arginine from 16 mg. in normal subjects to 127 mg. in our cystinuric patient, an average for a 24 hour period, and from 1.3 to 14.4% of the total amino acids measured; for cystine from 90 to 290 mg. and from 10.7 to 30.4% respectively; and for lysine from 17 to 230 mg. and 2.4 to 24.2% respectively. In hydrolyzed urines the corresponding increases were for arginine from 16 to 137 mg. for an average 24 hour period, and from 1.3 to 13.7% of the total amino acids measured; for cystine from 80 to 260 mg. and from 6.4 to 21.7% respectively; and for lysine from 60 to 250 mg. and from 5.2 to 23.7% respectively. The most significant average decreases were for glycine, from 690 to 260 mg. and from 68 to 23% in unhydrolyzed urines; and from 530 to 160 mg. and from 43 to 15% in hydrolyzed urines; for histidine, from 80 to 26 mg. and from 10.8 to 2.9% in unhydrolyzed urines; and from 90 to 38 mg. and from 7.7 to 2.8% in hydrolyzed urines. It is of further interest that the combined excretion of arginine, cystine, glutamic acid, histidine, glycine and lysine averaged about 90 (75.2 to 96.6) % of the 15 amino acids which were determined in all of the normal and cystinuric urines.

The data presented are in accord with the view that in cystinuric patients the metabolism of amino acids may be deranged in several respects and not only with regard to that of cystine. The report by early workers that the diamines, putrescine and cadaverine, which are closely related to arginine and lysine, were excreted in the urine by some cystinuric subjects is in harmony with this conclusion and with our own findings of an excreased excretion of arginine and lysine.

In confirmation of previous work,[4,5,6,11,14] ingestion of methionine increased urinary excretion of cystine in our patient.

Recently, Fanconi[8] called attention to the intimate pathogenetic relation of cystinuria to a particular syndrome characterized by stunted growth, rickets, hypophosphatemia, acidosis, glycosuria and high excretion of organic acids, including amino acids. It may be assumed that in cases showing this syndrome the disease develops first as cystinuria, leading to renal injury, followed by other metabolic changes characteristic for impairment of renal function (acidosis, hypophosphatemia, osteodystrophy). In this stage of

and daily on the days during which
† 12 hour sample.
‡ Values for hydrolyzed samples minimum since cystine and tyrosine are known to be partially destroyed by treatment with acid in the
oxygenated substances.
¶ Not determined since tryptophane is destroyed by treatment wih acid.

the progressive disease, cystine crystals may be found in organs and tissues, and demonstrated *in vivo* by bone marrow biopsy. Fanconi suggests the use of the terms "cystine storage disease," or with reference to the general disturbance of amino acid metabolism—"diabetes acid-aminicus et aminicus." Our patient has not shown the typical Fanconi syndrome, and the pathologic disturbance manifested itself only in the high excretion of cystine and several other amino acids. It is conceivable that the impairment of renal function with consecutive osteodystrophy will develop at a later age.

Summary. The daily excretion of 15 amino acids in unhydrolyzed and hydrolyzed normal and cystinuric female urines has been determined by microbiologic methods. Evidence has been presented which indicates that some amino acids are present largely in conjugated and some largely in unconjugated forms. Marked increases from the normal excretion of arginine, cystine and lysine and marked decreases in glycine and histidine occurred in cystinuria. The view of some early workers that there is a general disturbance in the metabolism of the amino acids in cystinuria is supported by the present observations.

REFERENCES

1. Abderhalden, E., and Schittenhelm, A.: Ztschr. f. physiol. Chem., **45**, 468, 1905.
2. Alsberg, C., and Folin, O.: Am. J. Physiol., **14**, 54, 1905.
3. Archibald, R. M., and Hamilton, P. B.: J. Biol. Chem., **150**, 155, 1943.
4. Brand, E., Block, R. J., Kassell, B., and Cahill, G. F.: J. Biol. Chem., **119**, 669, 1937.
5. Brand, E., and Cahill, G. F.: Proc. Soc. Exp. Biol. and Med., **31**, 348, 1247, 1934.
6. Brand, E., Cahill, G. F., and Harris, M. M.: J. Biol. Chem., **109**, 69, 1935.
7. Dunn, M. S., Camien, M. N., Shankman, S., and Block, H.: Archiv. Biochem., **13**, 207, 1947.
8. Fanconi, G.: Helvet. Pediat. Acta, **1**, 183, 1946.
9. Garrod, A. E., and Hurtley, W. H.: J. Physiol., **34**, 217, 1906.
10. Hac, L. R., and Snell, E. E.: J. Biol. Chem., **159**, 291, 1945.
11. Hess, W. C., and Sullivan, M. X.: J. Biol. Chem., **142**, 3, 1942; **143**, 545, 1942; **146**, 381, 1942; **149**, 543, 1943.
12. Hoppe-Seyler, F. A.: Ztschr. f. physiol. Chem., **214**, 267, 1933.
13. Ladenburg, A.: Ber. d. deutsch. chem. Gesellsch., **18**, 2957, 1885.
14. Lewis, H. B., Brown, B. H., and White, F. R.: J. Biol. Chem., **114**, 171, 1936.
15. Loewy, A., and Neuberg, C.: Ztschr. f. physiol. Chem., **43**, 338, 1904.
16. Looney, J. M., Berglund, H., and Graves, R. C.: J. Biol. Chem., **57**, 515, 1923.
17. Meisenheimer, J.: Ztschr. f. physiol. Chem., **114**, 241, 1921.
18. Thiele, F. H.: J. Physiol., **36**, 68, 1907–08.
19. Udranszky, L. V., and Baumann, E.: Ztschr. f. physiol. Chem., **15**, 77, 1891.
20. Williams, H. B., and Wolf, C. G. L.: J. Biol. Chem., **6**, 337, 1909.
21. Wolf, C. G. L., and Shaffer, P. A.: J. Biol. Chem., **4**, 439, 1908.

THE EXAMINATION OF THE HYPERTENSIVE PATIENT

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In an era of preventive medicine and specific therapy we face a generation of physicians in fervent quest of still another antihypertensive formula, of a bigger and better surgical antihypertensive procedure. It is difficult to find a remedy with which to undo the harm wrought by parental contempt of the laws of heredity. Thus far, essential hypertension remains a familial disease, irrevocably determined by the inheritance of a constitution productive of arterial hypertension, and void of a satisfactory solution.

Meanwhile, the clamor for such a final solution tends to override our sense of diagnostic discrimination in many an instance of hypertension arbitrarily designated as "essential." Occasionally, a patient, for years under supervision because of "essential hypertension," is finally recognized as having another clearly demonstrable disease. It is not known, of course, how many cases wrongly labeled "essential hypertension" never come to a correct diagnosis.

As it is, some physicians subject their hypertensive patients to a wide range of investigation, while many others do not go beyond using the sphygmomanometer. Between these two opposite diagnostic attitudes there is a wide gradation of attempt at differential diagnosis, more or less remote from completeness, depending upon the training, zeal, and inquisitiveness of the given physician.

Under these circumstances, it is deemed purposeful to outline a desirable routine examination of patients who manifest a significant rise of arterial tension. By the same token, it is proposed that the diagnosis of essential hypertension be made dependent upon evidence obtained from such routine examination. Patients not so examined or the cause of whose hypertension is not clearly definable should not be disposed of by the short-cut diagnosis of "essential hypertension;" they should be regarded as instances of arterial hypertension of unascertained origin.

Last but not least, extensive examination of the new patient afflicted with hypertensive disease serves a much broader purpose than mere etiological classification. It aids in the appraisal of the severity and spread of this disease, reveals its organic distribution and functional pathology, and facilitates prognosis and reasonable treatment.

The following is submitted as a feasible plan of investigation to be followed in cases of arterial hypertension. Several of these headings may offhand seem to be part and parcel of any complete examination. Under all of them, however, special points of pertinent interest are stressed apart from the usual routine which they imply: (1) Family history; (2) individual history; (3) physical examination; (4) urinalysis; (5) blood serology; (6) flat Roentgen ray plate of the chest; (7) electrocardiography; (8) nitroglycerine-test; (9) exercise tolerance test; (10) kidney function test; and (11) urography, and special urological investigation, if indicated by findings obtained from above procedures.

1. Family History. The family-tree is to be searched for the occurrence of hypertensive disease, vascular accidents, sudden deaths, eclampsia and allied hypertensive manifestations. The body build of the patient should be noted and compared, by careful questioning if necessary, with that of a parent or sibling known to be afflicted with, or to have died of, hypertensive disease. In this connection, the pyknic constitutional type merits particular importance: the short-necked, barrel-chested individual with well arched feet, high horizontal shoulder-girdle, good

muscular endowment, heavy skeletal frame.

The age of deceased parents is significant: patients with moderately severe hypertension may well reach the 6th or even the 7th decade of life; a record of decided longevity in the ancestry practically precludes the existence of grave hypertension of the familial type.

The discovery of one or more cases of congenital heart disease of any type in the family of a hypertensive patient should suggest the possibility of coarctation of the aorta with proximal arterial hypertension.

A dependable history of polycystic kidney in some member of the family prompts the consideration of a similar mechanism being involved in the arterial hypertension of the patient under examination and justifies instituting urographic and other measures for the study of such a diagnostic possibility.

2. Individual History. The past history and symptomatology of the young hypertensive patient need to be scrutinized with a view of possible presence of coarctation of the aorta: one may find parallel manifestations of "cold feet" and headaches in such patients, or the unexpected symptom of intermittent claudication, all explicable on the basis of the underlying anatomical variation of the arterial channels and their respective consequences above and below the coarctation.

Again, in the young hypertensive person, a history of "neuritis" attacks, unexplained cough, and bizarre episodes of abdominal pain, may well suggest a search for the generally elusive entity of periarteritis nodosa.

If the history is one of periodic acute episodes ranging from headaches to collapse, alternating with intervals of freedom from hypertensive complaints, and one is able to elicit also the complaint of unilateral lumbar backache, one should certainly not ignore the possibility of a pheochromocytoma with paroxysmal hypertension. Conversely, however, chronicity alone does not preclude such an impression. When one suspects the arterial hypertension to be due to such a medullary adrenal neoplasm, it may be helpful to question the patient concerning possible glycosuria in the past on some occasions but not on others: in such patients, simultaneous paroxysms of hypertension and excessive glycogenolytic bouts with glycosuria are likely to have occurred in the past.

The duration of the hypertensive history may be significant. If it coincides with menopause, spontaneous or surgical, and did not precede it by several years, one may suspect the existence of benign menopausal hypertension.

At times, a real and deliberate effort must be made to unearth a psychogenic cause of even very long lasting hypertension. Such an effort at probing the patient's history is especially indicated when the investigator is impressed with the contrast between the long duration and the remarkably benign course of the disorder. Occasionally, such search discloses a death, marital separation, or some other critical event in the patient's past ushering in the onset of complaints.

The importance of such findings in the history is appreciated when "essential" hypertension of seemingly serious severity actually ceases to exist after the provocative background has been eliminated. As an example of this kind the following case is cited.

Case Reports. Case 1. An obese woman 55 years old had a blood pressure up to 225/115, with severe hypertensive complaints of many years duration. She was married to a person not mentally normal, of a family studded with instances of insanity. An insane son born of this union lived at home. He had long been a source of emotional conflict between the patient's maternal attachment, on one hand, and her admission of the urgent need of his confinement at an institution, on the other. Also, her husband was steadily becoming a still further source of perpetual irritation and worry because of growing invalidism demanding constant attention. Significantly, her heart was not enlarged on Roentgen ray examination and

her electrocardiogram was satisfactory. After her husband died and her son finally was placed under institutional care, the patient, now living peacefully with other members of her family, has no hypertensive complaints, and her blood pressure averages 145/95.

One is always impressed with the history of years of anxiety and worry of the small business-man drifting hopelessly between poor credit, not enough stock, too few customers, too much chain-store competition, too many threats of foreclosure. Such an individual's elevated blood pressure, in the absence of definite evidence to the contrary, is not a manifestation of hypertensive disease; rather, it is a neurocirculatory reflection of his economic maladjustment.

In the history of such instances of "hypertension" one seeks, but does not find, any true episodes of hypertensive disease, such as stenocardia, anginal seizures, or paroxysmal nocturnal dyspnea. Conversely, one distinguishes from such real manifestations of hypertensive heart disease the various neurocirculatory complaints of "inability to breathe," the notorious sighing type of respiration, or the "nocturia" due to nervous insomnia but conspicuous by its absence during a spontaneous or induced restful night. Indeed, such patients often volunteer to supply a history of relative well-being during their hours of work and concentration, while their periods of distress, upon inquiry, may be found to follow excitement and aggravation.

On the opposite side of the ledger, a history of infections, no matter how remote in the patient's past and regardless of their severity, such as sore throat, tonsillitis, or scarlet fever, tentatively suggest the need of ruling out chronic glomerulonephritis with hypertension.

Lead poisoning in the patient's past is significant: it may be the source of hypertension encountered long afterwards, and the kidneys may never have been involved.[2]

Rare instances of "essential" hypertension are known to have been accounted for by head injuries and by cerebral infections which had occurred previously but which have produced neurological changes in the hypothalamus with resulting elevation of the blood pressure.

A history of diabetes mellitus militates against hasty labeling of patient's high blood pressure level as essential hypertension. In the first place, diabetics have a tendency to mild systolic hypertension;[3] in the second place, it is necessary to ascertain the patient's antidiabetic regimen and its efficacy. So many a diabetic person loses his "hypertension" after the diabetes has been brought under satisfactory control, without any other attempt at reducing the arterial tension.

3. Physical Examination. At the first examination, the finding of an elevated blood pressure should not be accepted with finality, in itself, as a token of hypertensive disease. This is clear in view of the wide range of blood pressure fluctuation under various circumstances and the emotional element often entering into consideration, especially during the first examination by a new physician. One proceeds examining the patient completely and, unable to elicit additional evidence of arterial hypertension, defers a conclusion until it is justified by several readings under basic conditions of physical rest and emotional equanimity.

On each occasion and as a matter of routine, the reflex elevation of the blood pressure following inflation of the cuff should be disregarded. Repeating the procedure slowly a few times, one generally finds the last reading the lowest.

An increased pulse rate detracts from the dependability of the blood pressure reading; it points to a momentary state of excitability with increased systolic arterial tension. This is encountered only too frequently and should not be confused with the picture of hyperthyroidism including tachycardia, systolic hypertension, relatively low diastolic blood pressure, and a wide pulse pressure.

Blood pressure determinations during

the menstrual cycle are often misleading. The same is true for the patient with a distended urinary bladder or one suppressing an urgent need of bowel evacuation. In cases of neurosis with vascular manifestations the blood pressure may be found to be appreciably high because the patient smoked before the examination.

Disparity of the blood pressure readings between the two arms, like the inequality of the radial pulse, should arouse the suspicion of luetic aortic disease or of extensive arteriosclerosis of the aortic arch with involvement of a subclavian artery.

In the young person, the discovery of arterial hypertension in the arms prompts one to examine the arterial circulation of the lower extremities: poorly palpable dorsal arteries of the feet and a blood pressure reading in the popliteal space paradoxically lower than that in the brachial arteries may lead to the diagnosis of coarctation of the aorta. Indeed, the palpability of the posterior tibial and dorsal arteries should be tested routinely in hypertensive patients of any age. In those suspected of having coarctation of the aorta, signs of compensatory collateral circulation should be looked for in the thorax, especially in the subscapular areas.

The physician need not regard this as bending backward to find a case of coarctation of the aorta. It is a fact that such cases are usually discovered accidentally by the roentgenologist, instead of by the clinician.

Case 2. A white woman, 42 years of age, came to the attention of the writer with the label of "essential" hypertension. She had been subject to episodes of bilateral headache and dizziness for many years. At one time, her 3rd pregnancy had been interrupted "therapeutically" because of hypertension, though 2 previous pregnancies had been entirely uneventful. Several fainting spells had occurred in the past. She complained of irritability, swelling of the ankles on prolonged standing, and increasing dyspnea on mild exertion. Strenuous exertion also caused substernal pain without radiation. During the time since November, 1943, she was examined by many observers. According to their records, her average blood pressure had been 195/105. Once, an episode of pyelitis prompted one of her examiners to raise the question of anatomical malformation of the urinary tract as a possible explanation of the entire picture, including the hypertension. The impalpability of the dorsal arteries of both feet had been noticed by one of her first attendants. Also, mild exophthalmus remained a constant finding, without any other signs of hyperthyroidism.

During the examination by this writer, the patient complained of choking sensations in recumbent posture, in addition to the symptoms referred to above. On that occasion, her blood pressure ranged from 235 to 240 systolic and from 95 to 105 diastolic. A review of the available clinical data seemed to establish clearly: marked systolic hypertension with but slightly elevated diastolic blood pressure in the absence of aortic insufficiency; uneventful course; appearance of good health despite the long duration of her hypertension; lack of any evidence in support of renal etiology; lack of any other clear-cut etiological data. The label of essential hypertension did not seem to be justified. On the other hand, the choking sensations on lying down, slightly wheezy expiratory breath sounds, the impression of increased dullness beneath the manubrium sterni, and the long known mild exophthalmus, suggested the possibility of a substernal thyroid adenoma. It was for this reason, that a basal metabolic rate determination and a chest Roentgen ray plate were obtained. The metabolic rate was found to be plus 22%, and there was no substernal thyroid lobe visible. However, the Roentgen ray plate showed a considerably widened first portion of the aorta, which may have been the cause of the increased dullness beneath the upper sternum, and very marked erosion of the inferior border of all ribs. Thus, the Roentgen ray findings, not the combined efforts of all clinical attendants, established the diagnosis of coarctation of the aorta.

When the examiner is impressed with considerable obesity in a patient whose blood pressure he found to be abnormally high, he will do well not to diagnose arterial hypertension unless the blood pressure remains high after adequate reduction of the patient's body weight. He

will, however, distinguish that type of hypertension which is associated with obesity of a characteristic distribution, hirsutism, abdominal striæ, and, possibly, other manifestations of Cushing's disease.

Precordial auscultation in a patient with high blood pressure often elicits the concomitant accentuation of the second aortic sound. During subsequent examinations, the blood pressure may, of course, vary from time to time. If the unduly loud second aortic sound remains constant, the persistence of this auscultatory observation, regardless of the variation in blood pressure level, connotes hypertensive disease, provided luetic aortitis and thyrotoxicosis have been ruled out.

No hypertensive patient is ever fully examined without visualization of the eye-grounds. Competent ophthalmoscopic examination, because of its diagnostic and prognostic importance, if not possible at the hands of the examining physician, should be requested elsewhere in each instance. In conjunction with other data derived from the study of each case, visualization of the eye-grounds may help in determining not only the extent of hypertensive vascular damage but also the pathogenetic type concerned. For a comprehensive review, the reader is referred to the corresponding chapter of A. Fishberg's "Hypertension and Nephritis."

4. Urinalysis significantly reveals nothing unusual in uncomplicated essential hypertension. When it discloses albumin in the urine, the possibility of orthostatic albuminuria should be ruled out. For the purpose of microscopic examination, a centrifuged urinary sediment should be obtained. It may provide a clue to the rôle, primary or otherwise, of the kidneys as well as to the extent of the renal disease. One stresses this point in view of the relatively small number of practicing physicians who resort to the use of a centrifuge.

Repeated urinalyses may occasionally produce findings suspicious of pyelonephritis requiring further examination and urological consultation. Information so obtained may lead to identification and removal of an infected kidney with abolition of the hypertensive mechanism.

5. Syphilis, cardiovascular or otherwise, is not known to play a causative rôle in arterial hypertension. Nevertheless, it is wise to institute serological tests in at least a great many cases of hypertension for the following reasons: The constitutional manifestation-time of hereditary hypertensive disease generally coincides with early middle age; so does the occurrence of luetic aortitis. Both produce, at first, the same complaints of stenocardia and mild dyspnea on exertion. In both, there is an accentuated second aortic sound. On the other hand, the height of the blood pressure in early arterial hypertension may not be impressive enough to be of decisive diagnostic aid. Under such circumstances, negative blood tests point to a hypertensive process, while positive results establish the existence of luetic aortitis. Needless to say, however, one occasionally sees both in the same patient.

6. Roentgen Ray Study of the Heart in Hypertension yields information concerning the size of that organ. In essential hypertension, as in cardiorenal disease due to primary chronic nephritis or nephrosclerosis, the left ventricle is enlarged. However, in primary Bright's disease the heart always increases in size; in essential hypertension, in relatively rare instances, it fails to show any significant change for a period of many years. There has never been any convincing explanation of this phenomenon. In the experience of the writer, this finding of a fairly normal cardiac silhouette in a case of true essential hypertension never justified any prognostic optimism: ultimately, the outlook proved to be commensurate with the sum total of all the other signs and symptoms of the disease. Such a finding does, however, dictate double caution in ascertaining whether or not one is confronted with some benign form of hypertension not productive of cardiac enlargement.

In hypertensive patients a Roentgen

ray plate of the chest is preferable to fluoroscopic examination. It is valuable by comparison with previous as well as with subsequent roentgenograms. Also, one is not likely to visualize fluoroscopically erosions of the ribs in coarctation of the aorta or inconspicuous foci of aortic sclerosis. For forensic purposes, too, the Roentgen ray plate is by far the more eloquent record.

7. An Electrocardiogram showing a moderate deviation of the electrical axis or mild slurring of the main deflections offers no aid in estimating the heart's share in the consequences of hypertensive disease. A high voltage electrocardiogram alone testifies, at times, to a long-lasting hypertension known to have a mortality[4] of about 40%. Extensive T-wave changes in the 1st and 2nd leads, in the absence of digitalis medication, gross alteration of the QRS complexes with delayed intraventricular conduction, or the electrocardiographic counterpart of pulsus alternans, indicate the existence of serious structural lesions and are not a good prognostic omen. Unfortunately, one meets altogether too often with a profuse indulgence in the stereotyped terminology of "myocardial damage" without any real foundation in the tracing; such loose "interpretation" must be discouraged.

Successive electrocardiographic records, like Roentgen ray plates taken at reasonable intervals, may be of considerable value in demonstrating consistently progressive changes of significance. The examiner will do well, therefore, always to compare a tracing taken of a new patient with a previously obtained electrocardiogram, if available.

8. Nitroglycerine Test. The reversibility of high blood pressure is naturally the object of immediate concern to the examiner. One used to hear in the past from some formidable clinicians how fortunate it was for the hypertensive patients that physicians did not have it in their power to lower their blood pressure. Yet, in the natural course of pathological events, such as following a cerebral or a cardiac vascular accident, one occasionally sees a patient with a lasting spontaneous reduction of the blood pressure not in the least to his harm. Conversely, there is good reason to expect vasospasticity of long duration to bring about in some vital organs a vise-like constriction of the vasa vasorum running along within the arteriolar walls, with impairment of nutrition and necrosis of arteriolar tissue.

For the purpose of testing the fixed or flexible nature of the patient's hypertensive level, the writer has, for several years past, routinely employed nitroglycerine sublingually, from 1/200 to 1/100 grain, depending on the body weight of the patient. No untoward effects have occurred. The method has the advantage of brevity. It obviates the need of resorting to more complicated depressor tests. It offers the same information within a period of about 5 minutes. When the patient is told, in advance, of the ensuing cerebral manifestations of the nitroglycerine effect, he accepts the assurance of their fleeting occurrence with equanimity.

9. Exercise Tolerance Test. For the purpose of testing the patient's exercise tolerance, no special devices are needed. With the blood pressure cuff attached to the arm, the patient is observed before and after brusque change from the supine to the sitting position repeated 25 times or less on the examining table. This procedure would seem to be simpler, but no less instructive, than hopping on the floor or negotiating a 3-step footstool.

10. Kidney Function Test. Ordinarily, it suffices to limit examining renal function to the concentration test and the estimation of the excretion of phenolsulphonephthalein. Both are easily carried out without the need of referring the patient to any institution or laboratory.

11. Urography. In the absence of any instance of hypertensive disease in the family, and without any objective evidence concerning the pathogenesis of the patient's sustained hypertension, extensive urological investigation is mandatory.

Ureteral catheterization should be employed in order to: (1) Secure urine from each side for microscopic and cultural studies; (2) test the secretion of phenolsulphonephthalein from each kidney; (3) obtain retrograde urograms. Probably, not enough coöperation between the physician in charge and the urologist has been cultivated hitherto for the benefit of obscure cases of arterial hypertension. The urologist's help is not requested often enough.

Summary. The finding of high blood pressure in a patient is a challenge to the examining physician. It imposes on him, in each case, the obligation to attempt etiologic study, classification, and overall appraisal of the pathologic process in its entirety.

For this purpose a suitable plan of bedside and laboratory examination is indispensable and should be adopted as a necessary routine. An outline of such examination is here discussed and proposed for consideration.

REFERENCES

1. Horine, E. F., and Weiss, M. M.: Am. Heart J., **6**, 91, 1939.
2. Lichtwitz, L.: Functional Pathology, N. Y., Grune & Stratton, p. 407, 1941.
3. Major, S. G.: Arch. Int. Med., **44**, 797, 1929.
4. Master, A. M.: Am. Heart J., **5**, 291, 1930.
5. Reid, W. D.: Ibid, **6**, 91, 1939.

INFARCTION OF AN ENTIRE PULMONARY LOBE WITH SUBSEQUENT ASEPTIC SOFTENING CAUSING STERILE HEMOPNEUMOTHORAX

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It is not generally realized that in rare instances pulmonary infarcts may undergo softening that is aseptic, with subsequent rupture into the pleural cavity, causing sterile pneumothorax. A search of the available literature has revealed only 5 such cases,[1,3,4,6] in only 3 of which the lesion was proven by postmortem examination.[3,4] To this small series we wish to add a sixth case, which shows several unusual features.

The reported cases are listed in Table 1. It will be seen that the proven cases all occurred in patients of about 40 years of age who suffered from heart disease and congestive failure. The source of the embolus was variable, as was the affected pulmonary lobe. The size of both infarct and perforation varied considerably. Perforation occurred in the 2nd or 3rd week following infarction. A positive pressure pneumothorax was the rule, and the character of the pleural fluid varied from serous to frankly sanguinous. In 4 cases,[3,4] including the one here discussed, the patients died shortly after the occurrence of the perforation; in the other 2 cases,[1,6] in both of which the etiology of the pneumothorax is open to question, the patients survived—at least until after the pneumothorax had disappeared.

Report of Case. A 39 year old colored male (Univ. of Penna. Hospital, No. '46–80, 142), whose family history was not significant, stated that he had been hospitalized at the age of 20 for 6 months for a severe illness, the nature of which is not known. Except for this episode he had enjoyed good health. One year prior to admission, he was rejected by the Army because of albuminuria. About this time he began to notice weakness, easy fatigability and weight loss. On Oct. 25, 1946, he was suddenly stricken with an attack of dyspnea while climbing a ladder. This forced him to stop work. Abdominal distention developed on November 15, and was accompanied by generalized abdominal pain and constipation. For the first time he noted ankle edema. The next day he was admitted to this hospital.

Physical examination at this time showed evidence of congestive heart failure, with venous distention, and coarse rhonchi and râles in both lungs. There was moderate ankle edema, and the liver extended 3 fingers below the costal margin. The heart was moderately enlarged, and the sounds were weak and muffled. The blood pressure was 114/90 mm. Hg. Respirations were 32 per minute. The pulse was 90, and its quality was poor. The patient was afebrile. There was some abdominal distention.

Laboratory examinations revealed a normal blood count, a negative Wassermann reaction, considerable albumin in the urine and occasional granular casts. Blood urea nitrogen was 16 mg. per 100 cc.; serum bilirubin was 1.6 mg. per 100 cc. The sedimentation rate was 33 mm. per hour, and electrocardiographic studies gave equivocal results.

Roentgen examination of the chest (Fig. 1) showed an enlarged heart with left ventricular preponderance, passive congestion of the lungs, and effusion or thickened pleura on the right side.

The patient was digitalized with improvement of all symptoms, and disappearance of the signs of congestive failure. Examination at this time disclosed an aortic diastolic murmur and a protodiastolic gallop. He seemed in excellent condition, how-

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ever, and was discharged on Dec. 7, 1946, to be followed as an out-patient.

He was seen 1 week later, and seemed to be doing well, but shortly thereafter edema recurred, and he stayed in bed at home. Three weeks before the second admission (about 3 months after leaving the hospital) he developed hemoptysis, followed by pain in the left chest, severe dyspnea, cough and increased weakness. He was readmitted on March 21, 1947. Hemoptysis was considerable, and pulmonary infarction was suspected. The patient was in marked congestive failure, and soon developed jaundice. The Van den Bergh test gave an immediate direct reaction, and serum bilirubin was found to be 6.8 mg. per 100 cc. The blood urea nitrogen rose progressively to 98 mg. per 100 cc. The right leg was more swollen than the left, but other signs of phlebothrombosis were absent.

Three days after admission, Roentgen examination showed signs of a left pneumothorax with collapse of the left lung, and the presence of fluid at the base. The mediastinum was shifted to the right. Areas of decreased density were seen in the region of the left lower lobe; the lower part of the right lung appeared consolidated and the remainder of the right lung markedly congested (Fig. 2). The pressure in the left chest was found to equal 700 mm. of water. On aspiration, 2000 cc. of air and 180 cc. of dark bloody fluid were removed.

The positive pressure pneumothorax was not controlled by repeated aspirations of the left chest or by supportive treatment. The patient died 4 days after admission, about 5 months after the clinical onset of congestive failure.

Autopsy. The body was that of a well-developed well-nourished colored male, with a moderate degree of jaundice. Both legs showed pitting edema extending to the groin, the right leg being considerably more swollen than the left.

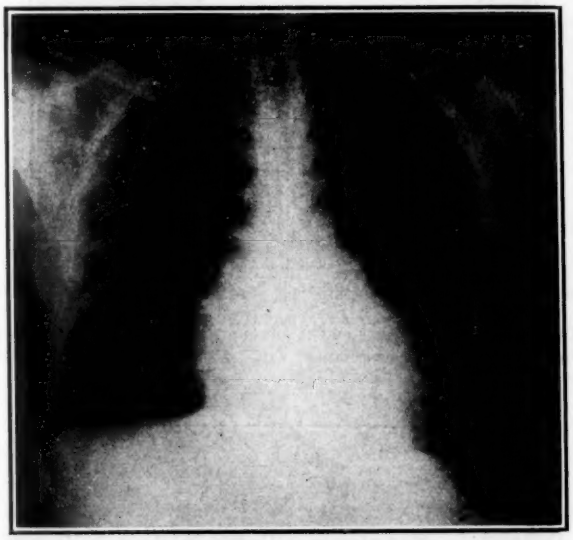

FIG. 1.—Roentgenogram of the chest on first admission. The heart is enlarged and shows left ventricular preponderance. The lungs exhibit slight evidence of congestion and there appears to be thickened pleura or effusion in the right pleural cavity.

The left pleural cavity contained air under tension, and 600 cc. of bloody fluid. The left lung was attached to the apical parietes by a dense fibrous band. The right lung was adherent on all sides by fibrous bands. The layers of the pericardium were thickened and everywhere firmly adherent.

The heart weighed 590 gm. The left ventricle was dilated and hypertrophied, and contained small old adherent mural thrombi. No evidence of myocardial infarction was noted, however. The chordæ tendinæ of the mitral valve were thickened and fused.

The aortic cusps were thickened and calcified and fused, and their free edges were rolled inward. The valves appeared to be both stenotic and insufficient.

The upper lobe of the left lung (Fig. 4) was almost completely collapsed. There was infarction of the entire left lower lobe caused by a large embolus, broken off thrombus, which completely occluded the principal artery to the lobe. The entire lobe was

FIG. 2.—Roentgenogram of the chest, shortly before death. There is collapse of the left lung associated with almost complete left pneumothorax. At the base of the left lung are several areas of decreased density. These proved to be due to air in the liquefied portions of the infarcted left lower lobe. There is a collection of pleural fluid on the left. The right lung is markedly congested and there are some areas of consolidation in the lower lung field.

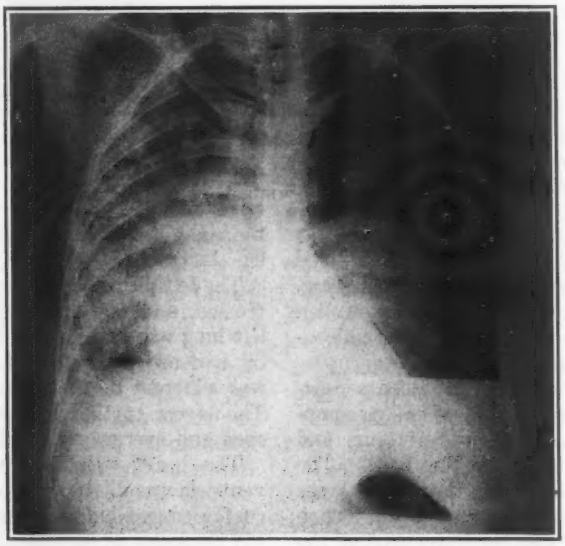

FIG. 3.—Roentgenogram of the chest taken after death. Note the mottled areas of decreased density n the left lower lobe which are due to air in the softened and ruptured parts of the lobe. Note fluid evel and shift of mediastinum to the right.

bulky, solid and dark blue-gray on its outer surface. The cut surface was firm and dark red in color. The lower and anterior portion of the lobe was occupied by a large ragged cavity, which communicated with the pleural space by a wide circular opening measuring 6 cm. in diameter. The interior of the cavity was filled with clotted blood and soft débris. Another, smaller cavity, 3 cm. in diameter, was found in the interior of the upper portion of the lobe, the remainder of which had the characteristic appearance of a hemorrhagic infarct.

Microscopic study of the various organs added no information to the gross examination. Of particular interest is the absence of bacteria in the softened areas of the pulmonary infarct, indicating its aseptic character.

Comment. A number of unusual features occur in this case, namely the large size of the pulmonary infarct, the aseptic character of the softening, its rupture into the pleural cavity with the causation of hemopneumothorax, the jaundice and the high blood urea nitrogen.

FIG. 4.—Left lung. Note aseptic softening of the infarcted lower lobe. A circular opening on the pleural surface, 6 cm. in diameter, leads into a ragged walled cavity in the left lower lobe, which is completely infarcted. The cavity is filled with débris and clotted blood.

The right lung contained several smaller infarcts in its middle and lower lobes respectively. The other organs showed passive congestion, which was very marked in the kidneys. In the right femoral and iliac veins was a large adherent antemortem thrombus, presumably the site of origin of the emboli.

It is well known that the size of pulmonary infarcts may vary "from a diameter of about 1 cm. to such a size as to occupy almost the whole of a lobe."[5] In this case the entire left lower lobe was

infarcted owing to occlusion of its main artery.

The ordinary course of events after the occurrence of pulmonary infarction (provided the patient survives a sufficient length of time) is gradual organization of the infarct resulting in a fibrous scar. In very rare instances, however, the necrotic area may undergo aseptic softening with resulting cavitation;[2,7] the present case is an example of this unusual outcome.

in the right base are rather typical of the appearance seen when infarcts occur in a markedly congested lung, as we have observed them in several similar studies by postmortem roentgenograms. The mottled areas of decreased density in the left upper lobe proved to be due to partly aërated lung. The similar but more sharply defined areas seen in the left lower lobe were due to air within the liquefied portion of this infarcted lobe.

TABLE 1.—ANALYSIS OF DATA OF REPORTED CASES

Case	Sex	Age	Underlying condition	Source of embolism	Location of infarct	Size of infarct	Size of perforation	Time of occurrence of perforation	Tension pneumothorax	Pleural fluid
Hayashi[4] No. 7	M	37	Cardiac infarct	Rt. vent.	RLL	Large	Large	..	Yes	Serofibrin.
Hayashi[4] No. 8	F	40	Nephritis, lft.vent. hypertr., rheum. mitral dis.	Leg	RUL	..	..	..	..	Serofibrin.
Guggenheim[3]	F	37	Coronary dis.	Leg (?)	R	Size of a nut	Size of a lentil	12 days	..	Serous
Daniels[1]	M	20	Typhoid	Leg	L	..*	..	..	Yes	None
Rogers[6]	M	44	Hyperten. ht. dis., card. infarct, apical aneurysm	Rt. vent.	RLL, LLL, RML	Largest, 4 x 4 x 3 cm.	..†	9 days	No	Serous
Rawson and Cocke	M	39	Rheum. aortic dis., femoral phlebothrombosis	Leg	LLL	Entire lobe	6 cm. (diam.)	3 weeks	Yes	Bloody

* No autopsy.
† Perforation not demonstrated at autopsy.

Jaundice is stated to be an occasional complication of pulmonary infarction, and apparently is a result of hemolysis within the infarct superimposed upon a liver functionally impaired by congestive failure.[2,7] The elevated urea nitrogen noted in this case probably can be explained upon the basis of the release of nitrogenous products from the infarcted area, and the functional impairment of the kidneys owing to chronic passive congestion.

The roentgenological features are of interest. The soft ill-defined character of the shadows cast by the recent infarcts

Summary. 1. A case is reported of a 39 year old colored male who developed complete infarction of the lower lobe of the left lung subsequent to long-continued passive congestion. The precipitating cause for the infarction was lodgement of an aseptic thrombus from the right iliac vein. The infarct rapidly underwent aseptic softening with consequent rupture into the pleural sac, leading to hemopneumothorax.

2. The course of events outlined is extremely rare, only 5 previous cases having been found in the world's literature.

REFERENCES

1. DANIELS, L. P.: Nederl. Tijdschr. v. Geneesk., **80**, 1455, 1936.
2. GSELL, O.: Deutsch. med. Wchnschr., **61**, 1317, 1935.
3. GUGGENHEIM, A.: Ann. d'anat. path., **12**, 872, 1936.
4. HAYASHI, J.: Frankf. Ztschr. f. Path., **16**, 1, 1915.
5. MACCALLUM, W. G.: A Textbook of Pathology, 6th ed., Philadelphia, W. B. Saunders, p. 37, 1937.
6. ROGERS, H. M.: Am. Heart J., **32**, 519, 1946.
7. SCHWEDEL, J. D.: Clinical Roentgenology of the Heart, New York, Paul B. Hoeber, 1946.

SIGNIFICANCE OF CLINICAL FINDINGS IN CIRRHOSIS OF THE LIVER

A Study of 93 Autopsied Cases

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This study is an analysis of cases of fatal Laennec cirrhosis of the liver that came to autopsy at the New York City Hospital in the 26 years from 1920 to 1945, with a comparison of the clinical and pathologic findings in the older and the more recent cases. Among 5916 autopsies reviewed, there were 93 cases in which the death could be directly attributed to cirrhosis. Patients whose hospital stay was less than 24 hours were not included, unless an admission diagnosis of Laennec cirrhosis had been made. The records were divided into 2 groups. Group 1 consisted of 37 cases, dying between 1920 and 1934; Group 2, of 56, between 1935 and 1945.

In both the earlier and the later group, the pathologic anatomic findings were those classically found in cirrhosis, frequently with fatty metamorphosis and superimposed acute hepatic damage. The spleens were enlarged, with increased fibrosis, perisplenitis or chronic congestion. Thrombosis of the portal vein was found in 1 case. Because of the similarity of the lesions in all these patients and adherence to the classical pictures, only the weights of the liver and spleen and the presence of collateral circulation were tabulated. In Group 1, the average weight of the livers was 1324 gm. and the mean weight of the spleens was 332 gm. There was anatomic evidence of collateral circulation in 35%. In Group 2, the livers averaged 1798 gm., the spleens 307 gm. and collateral circulation was found in 66%. These data are tabulated in Table 1.

Table 1.—Pathologic Findings

	Group 1	Group 2
Liver	1324 gm.	1798 gm.
Spleen	332 gm.	307 gm.
Collateral circulation	35%	66%

Race, Sex, Age. As might be expected in a city with a cosmopolitan population, the patients were of various backgrounds and occupations. As far as race is concerned, however, there were 7 Negroes and 86 whites, a ratio of 1:12. This is a smaller percentage than found by Kirschbaum and Shure,[4] who reported a ratio of 1:6. Moon's[6] report to the International Society for Geographic Pathology showed 2 whites to 1 Negro. The average age in the entire series was 54, the average age of both the earlier and the later group falling in the 6th decade. This agrees with the findings of most investigators that cirrhosis is a disease of middle life. Sex differences showed an increasing number of female cirrhotics in the later group. In the entire series, there were 39 females and 54 males. In Group 1, the ratio of males to females was 1.8:1; in Group 2, the ratio dropped to 1.1:1. These data are tabulated in Table 2.

Table 2.—Analysis of Race, Sex and Age

	Race		Sex		Age
	W	C	M	F	yrs.
Group 1	34	3	24	13	53.8
Group 2	52	4	30	26	54.1

Clinical Data. Evidence is steadily accumulating that dietary deficiencies are important factors in the etiology of hepatic cirrhosis.[2] In this series, the state of

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nutrition was compared in the 2 groups. It was found that in the earlier studied group, 23 patients (62%) were poorly nourished, whereas 17 (30%) of the second group were in a state of poor nutrition. The use of alcoholic beverages was likewise investigated. Of the total number, 61 were either heavy or moderate drinkers, 8 were teetotalers and the habits of the remaining 24 were unknown.

TABLE 3.—CLINICAL DATA—RANGE OF INCIDENCE IN %

	From history		Physical findings	
	Group 1	Group 2	Group 1	Group 2
Nutrition—Good	..	..	14–38	39–70
Poor	..	..	23–62	17–30
Ascites	22–59	32–57	29–78	42–75
Pain	19–51	26–46		
Dyspnea	13–35	17–30		
Weight loss	11–30	19–34		
Nausea and vomiting	14–30	24–43		
Pedal edema	16–43	19–34	13–35	22–39
Anorexia	11–30	23–41		
Jaundice	10–27	21–38	17–46	36–64
Weakness	11–30	21–38		
Hemorrhage	11–30	29–52	11–30	32–57
Collateral circulation—				
In vivo	..	..	15–41	24–43
At necropsy	..	..	13–35	41–66
Palpable liver	..	..	25–68	42–75
Palpable spleen	..	..	9–24	10–18

Of the other symptoms and objective findings noted in the clinical records, only those which occurred in more than 25% of cases were used for analysis. Except for cough, which was present in 18%, all the other findings were scattered items which could not be compared in the 2 groups. The most frequent complaint and the cause of hospitalization was ascites, presented by 58% of the entire series. Moreover, the incidence was nearly identical in both groups, 59% in Group 1 and 57% in Group 2. On physical examination, ascites was found even more frequently, 78% in the first and 75% in the second group.

The next most common complaint was abdominal pain or distress and was about equally distributed, 51% in Group 1 and 46% in Group 2. Dyspnea, loss of weight, nausea and vomiting occurred less often but in the same relative distribution in both groups. Pedal edema was more frequent in the early group. Loss of appetite, jaundice and weakness were present more frequently in the second than in the first group. Jaundice was more frequently found by observation, 46% of the early and 64% of the later group.

The most significant finding, however, was the difference in the occurrence of hemorrhage. Whereas only 30% of Group 1 complained of some hemorrhagic phenomenon, 52% in Group 2 had episodes of bleeding. This was further substantiated by the physical findings and at autopsy. Anatomic evidence of collateral circulation was found in 35% of Group 1 and 66% in Group 2.

A palpable liver and spleen were found in about the same proportion in both groups. The clinical data are summarized in Table 3.

Laboratory Data. Liver function tests have come into general use only in the last 15 years or so, therefore there are few data in this field in Group 1. The tests to be analyzed in Group 2 are the icterus index, serum cholesterol and cholesterol esters, and the serum albumin and globulin ratio. Of the other tests, there are insufficient numbers to warrant analysis.

The icterus index was determined in 37 cases. In 4 instances, the findings were normal, the highest figure was 260 and the average was 60.

The serum cholesterol and cholesterol esters were determined in 24 cases. The

total cholesterol values were normal (150 to 250 mg. %) in 8 instances, low (below 150 mg. %) in 14, and high (above 250 mg. %) in 2. The cholesterol esters were low (below 60% of the total) in 20 of the 24 cases (83%). Of the 4 cases with normal fractionation 2 had total cholesterol below normal limits, in 1 the total was above normal and in 1 both total and esters were normal. In 23 of the 24 cases, therefore, the cholesterol-ester results were of significance. These data are summarized in Table 4.

TABLE 4.—CHOLESTEROL–CHOLESTEROL ESTER FINDINGS

	Total cholesterol		
Esters	Low	High	Normal
Below 60% . . .	12	1	7
Above 60% . . .	2	1	1

The lowest normal limit for the albumin-globulin ratio regarded as normal in this institution is 1.3:1.6. In this series the test was performed on 21 patients. The average ratio was 1:1.1 and the average total protein 5.12 gm. In 14, the A–G ratio was either less than the lowest limit of normal or the total proteins were under 5.5 gm.

Blood urea nitrogen or non-protein and sugar levels were estimated in most of the entire series. The sugar levels were usually normal. In Group 1, the average urea nitrogen was 24.6 per 100 cc.; in Group 2, the non-protein nitrogen was 35 mg. per 100 cc.

Erythrocyte counts were done in 29 instances. Only 5 were above 4 million. Of the 34 hemoglobin determinations, 26 were below 12 gm. Fifteen of 26 patients had a color index above 1. Only 7 of 30 patients had a white blood count below 10,000.

Discussion. The early group, from 1920–1934, represents a more advanced stage of cirrhosis than the later group of 1935–45. The livers were smaller than the normal weight of 1450 to 1750 gm. given by Piersol[7] and the state of nutrition was worse. The reason for this difference in the 2 groups is not apparent.

It is of interest to compare some findings in the 2 groups. Ascites was the most common presenting symptom in each group, occurred in the same relative proportion and its presence was equally demonstrable by physical examination. Three of every 4 had ascitic fluid when first observed. Lucké[5] also found in a study of early fatal epidemic hepatitis that ascites was a common finding. Ascites, therefore, tends to occur early in the disease and is not, as the older teaching held, a late manifestation.

The mechanism of its production is complex and still a matter of controversy. It is generally said that it is due to a diminution of the colloid osmotic pressure following a change in the plasma proteins, and also to portal hypertension following increasing obstruction to the blood flow through the liver. In the present series, the protein level was determined in 21 ascitic cases. In 14 (67%) the A–G ratio was less than 1.3:1 or the total proteins were less than 5.5 gm. In the same group, 20 of the 21 cases (95%) had either an enlarged spleen or anatomic evidence of collateral circulation. In the entire series, 71 had ascites and 59 of them had splenomegaly. Of the remaining 12 with small spleens, 4 had varices. In other words, 90% of all the ascitic cases from 1920–1945 had evidence of portal hypertension and obstruction to the hepatic circulation. In Lucké's series, ascites often appeared before a drop in the total proteins and the spleens in the ascitic cases were enlarged twice as often as in the non-ascitic. Several workers have also shown that there is no constant relationship between colloidal osmotic pressure and the level of the plasma protein.[1] It is justifiable, therefore, to conclude that portal hypertension has a greater influence in the production of the ascites than does hypoproteinemia.

Jaundice was frequent and sometimes severe. As an early complaint, it was somewhat more common in the second group, but objectively it was very common, was found in almost half of Group 1 and two-thirds of Group 2. The higher

levels were always associated with a greater degree of acute hepatic damage.

The greatly increased incidence of hemorrhage in Group 2 is difficult to explain, but this feature has been noted by other observers.

Of the 3 liver function tests, the most delicate appeared to be the cholesterol-ester fractionation. It was disturbed, with a drop in the ester component, in 96% of the cases. It gave evidence of hepatic damage at an earlier stage than either the albumin-globulin ratio or the icterus index.

Summary and Conclusions. 1. Ninety-three autopsied cases of fatal Laennec cirrhosis of the liver are analyzed. They were divided into 2 groups: one, of 1920–1934, presenting a late stage of cirrhosis, and a second, of 1935–1945, presenting an earlier stage.

2. Ascites was as common in the early as in the late stage of cirrhosis and appears due to obstruction of the hepatic circulation rather than due to hypoproteinemia.

3. In the more recent group presenting excessive stages of cirrhosis, hemorrhage was found much more frequent than in the group representing an older cirrhotic process, for reasons that were not explained.

4. The estimation of the cholesterol fractionation was one of the more delicate tests of hepatic function.

REFERENCES

1. Butt, H. R., Snell, A. M., and Key, A.: Arch. Int. Med., **63**, 143, 1939.
2. György, P.: Am. J. Clin. Path., **14**, 67, 1944.
3. Karsner, H.: Am. J. Clin. Path., **13**, 569, 1943.
4. Kirschbaum, J. D., and Shure, N.: Quoted by Karsner, Ref. 1.
5. Lucké, B.: Am. J. Path., **20**, 471, 1944.
6. Moon, V. H.: Quoted by Karsner, Ref. 1.
7. Piersol, G. A.: Human Anatomy, Phila., Lippincott, p. 1706, 1907.

INTERFERENCE BETWEEN INACTIVE AND ACTIVE VIRUSES OF INFLUENZA*

V. Effect of Irradiated Virus on the Host Cells

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In previous communications from this laboratory[10–14] it has been reported that injection of inactivated influenza virus into the allantoic sac of the chick embryo may prevent the propagation of subsequently injected active virus. Similar observations have been made by others.[25] In these studies the conditions for the optimal production of interfering preparations have been analyzed.[11] Irradiation by ultraviolet light gave more consistent results than heating to 56° C. or formalinization of the virus and the former procedure was therefore adopted for further studies.[12] It was found that the reaction is non-specific in that it occurred between the serologically distinct types of influenza A and B,[13,26] as well as between the influenza viruses and biologically non-related agents such as the viruses of western equine encephalomyelitis,[13] epidemic keratoconjunctivitis[13] and mumps.[15] It was shown by various physical and serologic means[14] that the interfering agent was identical with the inactivated virus particle. In these various studies little or no information has been gathered in regard to the reactions of the host cells. In the present paper some experiments will be related which tend to fill this gap. They were concerned with the localization of the interference reaction, the speed with which the changes are induced and the duration of the altered stage, the effect of irradiated virus on the development of the host and the adsorption of the inactivated agents on to the host cells.

Methods and Materials. The essential techniques employed in the interference experiments have been described in detail in previous papers of this series.[11–14] The technique of the hemagglutination test has been modified during the course of these experiments. In the earlier tests the method of Hirst and Pickels[20] was used; in later experiments the "pattern test" was employed as described previously.[17] For the experiments on the adsorption of hemagglutinin on to the cells of the allantoic sac the technique of Miller and Stanley[24] was adopted.

Experimental. *Localization of the Interference Reaction.* It is generally felt that the interference phenomenon is caused by certain changes either on or within the host cells due to direct contact of the cell with the interfering agent. The conditions encountered in the chick embryo permitted a test of this hypothesis and the results reported below serve to substantiate its validity. It was found that injection into the allantoic sac of infected allantoic fluid which had been dialyzed and irradiated by ultraviolet light for 3 minutes produced interference only within this structure, whereas the amniotic cavity remained fully susceptible to infection by active influenza viruses. The reverse experiment, the introduction of irradiated virus into the

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amnion with subsequent infection by the amniotic or allantoic routes, gave less clear-cut results. This difference in the results was caused by the fact that injection into the amnion can be made only by passing the needle through the allantoic cavity. This leaves an open communication between the 2 cavities which permits subsequent exchange of either irradiated or active virus. When these effects were minimized by using amounts of the agents close to the minimal effective doses the results become more uniform. A summary of several experiments is presented in Table 1. They show, then, that interference is noted only locally and no generalized protection is obtained by this means.

A simpler, and perhaps more accurate, method was used in later experiments. This involved the use of potent homologous immune serum, against the PR8 strain, for instance, for the interruption of the process of adsorption on to the host cells of the homologous irradiated virus. The state of interference was then measured by the injection of active heterologous virus such as the Lee strain. An experiment of this type is shown in Table 2. As can be seen, 0.2 ml. of anti-PR8 immune rabbit serum injected simultaneously with 0.5 ml. of irradiated PR8 virus prevented interference with the active Lee virus (0.2 ml. of a 10^{-5} dilution) given 2 hours later. Normal serum did

TABLE 1.—THE INTERFERING EFFECT CAUSED BY IRRADIATED INFLUENZA VIRUS—A LOCALIZED ONE ONLY: A SUMMARY OF 3 EXPERIMENTS

1st injection		2nd injection active virus route	Results of hemagglutination test 48 hours after 2nd injection	
Material	Route		Allantoic fluid	Amniotic fluid
None	..	Allantoic	Positive	Negative
		Amniotic	Positive*	Positive
Normal allantoic fluid irradiated for 3 minutes	Allantoic	Allantoic	Positive	Negative
		Amniotic	Positive*	Positive
	Amniotic	Allantoic	Positive	Sometimes positive†
		Amniotic	Positive*	Positive
Infected allantoic fluid irradiated for 3 minutes	Allantoic	Allantoic	Negative	Negative
		Amniotic	Negative	Positive
	Amniotic	Allantoic	Usually positive‡	Negative
		Amniotic	Usually negative*‡	Negative

* Contaminated needle passes through allantoic sac in order to reach amniotic sac; this deposits enough virus to cause infection in practically all allantoic sacs.

† Infection from allantoic sac through hole left by first injection into amniotic sac.

‡ A few negative reactions possibly caused by backflow of irradiated virus from amniotic sac into allantoic cavity.

The Speed With Which Interference is Induced. It has been shown previously that interference may be induced rather rapidly in the allantoic sac of the chick embryo.[12] When 12 day old embryos were injected with 1 ml. amounts of irradiated virus while the allantoic sac was flushed with buffered saline solution at a rate of about 15 to 20 ml. per minute for 10 to 20 minutes, the tissue was found to be resistant to subsequent infection with active virus even though the contact of the host cells with the irradiated virus has been very brief.

not affect the interference reaction. When the immune serum was given 1 minute after the irradiated virus the interfering agent was no longer intercepted, so that the subsequently administered active influenza B virus failed to propagate sufficiently to develop measurable quantities of hemagglutinins. In another experiment, the interfering activity was still partially intercepted by serum injected 1 minute after the irradiated virus and only partial interference was obtained. In the same experiment, however, the serum given after 3 minutes failed to prevent interfer-

ence in that experiment. The hemagglutinin titers of 1:64 found by the pattern test in the groups injected with irradiated virus followed by normal serum represented residual, non-adsorbed irradiated virus, since the controls not injected with Lee virus showed the same titers. In addition identification of these hemagglutinins with known immune sera proved them to be of Type A. These residual hemagglutinins were neutralized in the groups of eggs injected with the anti-PR8 immune serum. This serum in itself did not inhibit the growth of the Lee virus, as can be seen in the results obtained in control eggs primarily injected with normal allantoic fluid. These data confirm the earlier observations in that they demonstrate that interference is induced rather rapidly, *i. e.*, within 1 minute or less.

Duration of the State of Interference. By means of a technique similar to that described in the previous section it could be determined how long the host cells were resistant to infection after contact with irradiated virus. In former experiments,[12] irradiated virus was injected into the allantoic sac of 8 day old embryos and the active test virus was given up to 96 hours after the primary injection. In these experiments not all the irradiated virus was adsorbed by the host cells; some was left free in the allantoic fluid of the injected egg. It was conceivable that such amounts of residual, non-adsorbed virus could account for the prolonged protection. Therefore, these experiments were modified as follows: 0.5 ml. of irradiated allantoic fluid (PR8) was injected; after 1 hour, 0.2 ml. of specific immune serum (anti-PR8) was injected to neutralize the unadsorbed particles of virus remaining from the first injection. When eggs thus treated were infected with 0.2 ml. of a 10^{-5} dilution of active heterologous virus (Lee) 2 to 6 days later, it was seen that no measurable concentrations of hemagglutinin were produced (Table 3). In other words, the cells of the allantoic sac were resistant to infection for at least 6 days following treatment with the irradiated virus. It was found impossible to test for resistance after longer intervals since the amount and composition of the allantoic fluid changes markedly after the 15th day of total incubation of the chick embryo.

TABLE 2.—SPEED OF PROTECTION BY INTERFERENCE

	2nd injection		Results of 3rd injection (2 hours after 1st injection)					
			Active Lee virus			Control		
1st injection, interfering agent	Rabbit serum	Interval between 1st and 2nd injection (min.)	No. of eggs positive	Hemaggl. titer of pool	Type virus in pool	No. of eggs positive	Hemaggl. titer of pool	Type virus in pool
PR8 allantoic fluid irradiated for 3 minutes	Anti-PR8	0	9/9†	1:1024	B	0/5	<1:2	
		1	0/8	<1:2	..	0/5	<1:2	
		60	0/6	<1:2	..	0/4	<1:2	
	Normal	0	8/9	1:64*	A	4/5	1:64*	A
		1	7/7	1:64	A	4/5	1:64	A
		60	5/6	1:64	A	2/2	1:64	A
	None	..	7/7	1:64	A	4/5	1:64	A
Normal allantoic fluid irradiated for 3 minutes	Anti-PR8	60	6/6	1:1024	B	0/4	<1:2	
	Normal	60	6/6	1:2048	B	0/4	<1:2	
	None	..	6/6	1:2048	B	0/5	<1:2	
None	None	..	8/8	1:2048	B			

* Residual hemagglutinins from first injection.
† 9 out of 9 allantoic fluids positive.

Effect of the Interfering Virus on the Functions of the Host Cell. In the course of these experiments 8 day old chick embryos were employed in a number of instances. It was noted in some of these cases that following injection of the more potent irradiated preparations of virus

the embryonic development slowed down. This phenomenon was studied further. A suitable number of 8 day old embryos were candled and the extent of the air sac and of the shadow of the allantoic sac were marked by pencil. After injection of 1 ml. of irradiated virus or normal allantoic fluid, likewise exposed to ultraviolet light, the eggs were returned to the incubator and candled daily for 8 days to measure the further development of the allantoic sac. At the end of the experimental period the increase of shadow developed during the 8 days of incubation was blackened and the eggs were photographed as shown in Figure 1. As can be seen the shadow in the eggs injected with normal allantoic fluid almost had reached the tip of the eggs at the end of the experimental period. On the other hand, those embryos injected with the irradiated virus showed only small increments in shadow and this increase had occurred usually during the first 2 days following the injection. In other experiments, using embryos older than 8 days at the onset, the slight additional growth of allantoic membrane noted in the first 2 days after injection of the irradiated virus usually sufficed to bring the shadow of the allantoic sac down to the tip of the egg. In the test shown in Figure 1 only 2 of the embryos in the experimental group had died during the period of the test, none in the group injected with normal fluid. The death rate of the injected embryos in similar experiments was usually of this order. In

TABLE 3.—PERSISTENCE OF RESISTANCE INDUCED BY INTERFERENCE

1st injection, interfering agent	2nd injection: Rabbit serum 1 hour after interfering agent	2nd injection: Interval between 1st and 3rd injection (days)	Result of 3rd injection: Active Lee virus: No. of eggs positive	Active Lee virus: Hemaggl. titer of pool	Active Lee virus: Type virus in pool	Control: No. of eggs positive	Control: Hemaggl. titer of pool	Control: Type virus in pool
PR8 allantoic fluid irradiated for 3 minutes	Anti-PR8	2	0/8†	<1:2	..	0/4	<1:2	
		4	0/7	<1:2	..	0/4	<1:2	
		6	0/10	<1:2	..	0/7	<1:2	
	Normal	2	5/6	1:4*	A	2/6	1:4*	A
Normal allantoic fluid irradiated for 3 minutes	Anti-PR8	2	7/7	1:512	B	0/4	<1:2	
		4	8/8	1:768	B	0/3	<1:2	
		6	9/10	1:384	B	0/4	<1:2	
None	None	2	8/8	1:512	B			
		4	8/8	1:512	B			
		6	10/10	1:384	B			

* Residual hemagglutinins from 1st injection.
† 0 out of 8 allantoic fluids positive.

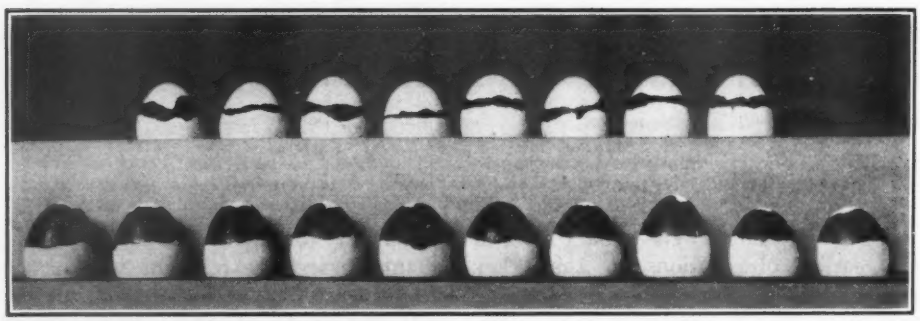

FIG. 1.—Increment in shadow of the allantoic sac in embryos injected with irradiated PR8 virus (upper row) and with irradiated normal allantoic fluid (lower row). The tips are pointing upwards.

no instance have the experiments been carried to hatching time.

Attempts were made to substantiate these observations in this and further experiments by weighing of the wet allantoic sacs of the 2 groups of embryos. However, the figures obtained were found to be not very consistent. They depended on the age of the embryo at the start of the experiment. The results were variable on account of the difficulties encountered in separating the allantoic and amniotic tissues and in removing small traces of allantoic fluid or saline solution used for washing which were caught in pockets of the membrane. In addition the allantoic sac of the eggs injected with irradiated material sometimes appeared quite edematous. A more consistent measure of the inhibitory effect was obtained when the shell of the tip of the eggs not covered by the shadow of the allantoic sac was weighed, but in that case differences in the thickness of the shell and loss of small fragments of shell during cutting were responsible for some inaccuracies. However, since 8 to 10 eggs were used per group and since the differences encountered in these experiments between the various groups were fairly large it was felt that these sources of error did not affect materially the interpretation of the results, and this technique was adopted, therefore, to obtain some measure of the degree of inhibition of growth. It was found, in addition, that the weight of the chick in the group inoculated with inactivated virus, was reduced as compared with that of the control group inoculated with normal fluid. In eggs of more than 8 days incubation at the time of injection of irradiated virus, the only consistent difference between the 2 groups was in the weight of the embryo. Table 4 summarizes a number of experiments showing a marked reduction of embryonic development by the various methods employed. In addition it can be seen that the amount of allantoic fluid remained at a high level in the inhibited eggs whereas the quantities decreased markedly in the controls.

TABLE 4.—EFFECT OF IRRADIATED VIRUS ON EMBRYONIC DEVELOPMENT

Inoculum (irradiated allantoic fluid)		Period of observation	Yield of embryonic materials (average of 8 to 10 eggs per observation)			
Strain of virus	Interference titer*	Age of embryos (days)	Allantoic membrane (gm.)	Shell of tip (gm.)	Embryo (gm.)	Allantoic fluid (ml.)
PR8	1:81	8–16	1.3	1.63	11.0	n.t.
normal		8–16	1.44	0.26	15.4	n.t.
PR8	1:81	8–17	1.4	2.1	12.3	n.t.
normal		8–17	1.7	0.27	16.6	n.t.
PR8	1:140	8–16	0.82	1.2	11.3	7.1
normal		8–16	1.84	0.25	15.4	2.75
Lee	n.t.†	8–16	0 66	1.66	6.3	8.0
normal		8–16	2 0	0 45	10.8	2.0
Lee	1:27	8–16	1.7	1.1	9.4	8.2
normal		8–16	1.66	0.7	12.1	4.3
Lee	1:81	8–16	n.t.	1.65	9.3	n.t.
normal		8–16	n.t.	0.62	12.6	n.t.

* Dilution of irradiated allantoic fluid preventing formation of measurable amounts of hemagglutinins in 50% of the eggs.
† Not tested.

One series of experiments served to establish the fact that only allantoic fluid irradiated for relatively short periods of time gave this inhibition of growth. As seen in Table 5, infected allantoic fluids irradiated for 3 to 5 minutes gave marked inhibition. When exposure for 15 minutes was used inhibition was obtained only occasionally and with longer irradiation

the phenomenon was no longer observed. It is to be noted that these results of inactivation of the growth inhibitor coincide with the inactivation of the interfering property of allantoic fluid. Maximal interference was obtained with fluids irradiated for 1 to 3 minutes, little if any with preparations exposed for 15 minutes and no effect was obtained after an exposure of 60 minutes.[11,16]

ence, on the other hand, could be demonstrated usually when using higher dilutions of irradiated allantoic fluid, *i. e.*, 1:81 or even 1:243. However, the absence of measurable concentrations of hemagglutinins at the end of the experimental period of an interference test does not exclude the propagation of some virus. It indicates merely that the number of susceptible host cells was sufficiently reduced so

TABLE 5.—EFFECT OF PROLONGED IRRADIATION ON GROWTH INHIBITOR

Inoculum (irradiated allantoic fluid)		Yield of embryonic materials (average of 8 to 10 eggs per observation)			
Strain of virus	Irradiation (min.)	Allantoic membrane (gm.)	Shell of tip (gm.)	Embryo (gm.)	Allantoic fluid (ml.)
PR8	3	0.86	0.90	9.0	7.7
	5	0.70	1.26	7.7	4.5
	15	1.24	0.27	11.9	4.0
	60	1.43	0.30	11.1	5.5
	120	1.20	0.60	12.3	5.8
Control	..	1.65	0.37	11.4	4.5
Lee	5	0.66	1.66	6.3	8.0
	15	1.27	1.05	9.0	3.0
	120	1.78	0.39	11.9	4.1
Control	..	2.0	0.45	10.8	2.0

TABLE 6.—EFFECT OF DILUTION ON GROWTH INHIBITOR

Inoculum (irradiated allantoic fluid)		Yield of embryonic materials (average of 8 to 10 eggs per observation)			
Strain of virus	Dilution	Allantoic membrane (gm.)	Tip of shell (gm.)	Embryo (gm.)	Allantoic fluid (ml.)
PR8	Undil.	0.86	0.90	9.0	7.7
	1:5	0.90	1.43	7.8	5.2
	1:10	1.48	0.96	9.0	7.4
	1:20	1.45	1.16	9.3	7.0
Control	..	1.65	0.37	10.3	4.5
PR8	1:10	1.33	1.43	10.7	5.9
	1:20	..*	0.63	11.7	6.5
	1:30	1.60	0.81	14.1	2.8
	1:40	1.96	0.55	15.3	3.6
	1:80	1.95	0.56	15.1	4.4
Control	..	1.55	0.49	14.8	3.4
Lee	Undil.	0.66	1.66	6.3	8.0
	1:10	1.0	1.13	9.8	6.3
Control	..	2.0	0.45	10.8	2.0

* Lost by accident.

The inhibitory activity could be noted only with more concentrated preparations of allantoic fluid. As can be seen in Table 6, dilution of the fluid to 1:10 or 1:20, *i. e.*, 0.1 to 0.05 ml. of allantoic fluid, may still produce inhibition of embryonic development. Higher dilutions failed to do so. Some degree of interfer-

that the amount of virus produced was insufficient to cause agglutination of red cells, *i. e.*, the infectivity titer was less than 10^{-7}.

The fact that not only the allantoic sac but also the chick embryos were retarded in their growth deserved further consideration. This result could have 2 possible

explanations: (*a*) the decreased growth of the membrane may affect in turn the embryo on account of an insufficient gaseous exchange through the limited expansion of the blood-vessels of the allantoic sac and through the inhibition of other physiologic function of this structure; or (*b*) the irradiated virus may affect the embryo first and subsequently the development of the allantoic sac. This question was studied in greater detail. First of all, resorption of materials from the allantoic sac appears slow and possibly limited to substances of low molecular weight.[22] As will be shown in the subsequent section of this paper, after an initial loss of hemagglutinin from the inoculum through adsorption on to the host cells the amount of residual virus particles in the allantoic fluid of the injected eggs remains fairly constant, suggesting that no further adsorption or resorption takes place. Another approach to this problem was the determination of membrane and embryo weights at daily intervals following the injection of the irradiated virus. The data obtained suggested that the membrane showed a reduction in growth within 48 hours before significant changes in the rate of the embryonic development were noted. Finally, experiments employing homologous immune chicken serum gave results comparable to those reported in a preceding section of this paper. If the immune serum was given simultaneously with the irradiated virus the inhibitor was neutralized as well as the interfering activity and the development of the embryo was unimpaired. However, when the serum was given 1 to 5 minutes after the irradiated virus so that the antibodies no longer intercepted the interfering activity, the inhibitory effect on the development of the host could likewise no longer be neutralized. Since all free irradiated virus in the allantoic fluid was neutralized by the serum it is strongly indicated that the inhibition of growth is produced by the contact of the irradiated virus particle with the allantoic cells and that the effect on the embryo is secondary to the inhibition of the growth of the membrane.

The experiments reported in this section show, then, that irradiated virus may exert profound effects on the host cells leading to an impairment of the development of the allantoic sac and in turn of the embryo itself. On prolonged irradiation of the infected allantoic fluids this effect is destroyed, even though the virus particle still may cause hemagglutination and may still be adsorbed on to red cells and on to the cells of the allantoic sac as will be recorded in the next section.

Adsorption of Virus Hemagglutinin on Host Cells. It has been shown by Hirst[18] that influenza virus is adsorbed on to red cells and cells of the respiratory tract of the ferret.[19] If the cells support the growth of the virus as in the case of the lung tissue of the living ferret, the virus is not eluted but apparently enters the cells and multiplies. No elution has been noted in chick embryos whose allantoic sac was flushed with saline solution after injection of irradiated allantoic fluid virus,[12] since no hemagglutinin was found in the fluid content of the allantoic sac after an incubation of the eggs for several days. On the other hand, if the host cells do not support the propagation of the virus as in the case of the red cells or the cells of the excised respiratory tract of the ferret, the virus elutes after adsorption.[18,19] The elution of the virus from the red cells is accompanied by changes in the red cells affecting their agglutinability by fresh influenza virus. This has been explained by the loss of "receptor spots" to which the virus attaches itself.[4,18] These receptors are presumably lost or destroyed as a result of the virus action.

Upon injection of known amounts of hemagglutinins into the allantoic sac of 10 to 12 day old chick embryos and collection of the allantoic fluids from the injected eggs as completely as possible after a certain time interval, hemagglutinins were recovered free in the fluid. However, there was always a deficit between the amount injected and the quan-

tity recovered. This missing amount represented, presumably, the amount of hemagglutinin adsorbed on to the cells lining the allantoic sac. The accuracy of the results obtained by this technique depended on 2 factors: the completeness of the collection of the allantoic fluid and the sensitivity of the hemagglutination test. As to the former factor, small quantities of allantoic fluid will be retained in folds of the sac, and care has to be exercised not to break the amnion. The accuracy of the hemagglutination test was somewhat increased in later tests by adoption of the technique of Miller and Stanley[24] who considered differences in titer of about 10% as significant. In spite of these difficulties it is felt that the data to be presented are sufficient to support the points to be made.

The results of tests conducted with a number of different preparations of infected irradiated allantoic fluid are summarized in Table 7. If the amount of hemagglutinin injected fell below a certain level, the collected fluids were found to give negative results in the hemagglutination tests. In the various experiments conducted with sufficient concentrations of hemagglutinin it was found that the amount adsorbed increased with the quantity injected. The adsorption amounted to 30 to 90% of the inoculum in these tests. When graded amounts of a given preparation of irradiated virus were injected, it could be shown (Table 8) that an inverse relationship existed between the quantity of hemagglutinin injected and the percentage adsorbed. With the injection of increasingly larger amounts of virus the percentage of hemagglutinins adsorbed decreased. The experiments

TABLE 7.—ADSORPTION OF HEMAGGLUTININ ON TO THE CELLS OF THE ALLANTOIC SAC

Strain of irradiated virus	Time of harvest (hrs.)	Hemaggl. (units injected)	Hemaggl. (units recovered)	Hemaggl. (units adsorbed)	% adsorbed
PR8	3	512	262	250	49
	6	512	221	291	57
	4	256	86	170	66
	2	256	102	154	60
	2	198	118	80	40
	0.5	192	131	61	32
	2	180	95	85	47
	2	78.5	29	49.5	64
Lee	3	1068	818	250	23
	2	128	13	115	90
	3	128	55	73	57
	2	86	35	51	59
	2	86	38	48	55
	2	38.5	13	25.5	66

TABLE 8.—EFFECT OF CONCENTRATION OF HEMAGGLUTININ ON ADSORPTION ON TO ALLANTOIC SAC

Strain of irradiated virus	Hemaggl. (units injected)	Hemaggl. (units recovered)	Hemaggl. (units adsorbed)	% adsorbed
PR8	180	95	85	47
	90	54	36	40
	45	17	28	62
PR8	192	131	61	32
	96	50	46	48
	48	10	38	79
Lee	1068	818	250	23
concentrated	534	319	215	40
4×	267	95	172	64
	134	62	72	54
	67	16	51	76

have not been carried far enough to reveal definitely whether a saturation point may be reached, *i. e.*, whether the injection of hemagglutinins beyond a certain concentration will not result in further additional adsorption. However, tests suggesting that this may be the case will be briefly discussed at the end of this section.

allantoic fluids of that age altered the hemagglutinating capacity of the agent as well as the pattern formed by the agglutinated cells in the bottom of the test tube.

The adsorption of virus on to the cells is independent of the interfering capacity of the virus. Whereas the property of interference is destroyed by irradiation for

TABLE 9.—INFLUENCE OF INCUBATION PERIOD ON ADSORPTION OF HEMAGGLUTININS

Strain of irradiated virus	Hemaggl. (units injected)	Time of harvest	Hemaggl. (units recovered)	Hemaggl. (units adsorbed)	% adsorbed
PR8	96	5 min.	73	23	24
		30 min.	63	33	34
		1 hr.	65	31	32
		2 hr.	61	35	36
		4 hr.	62	34	35
		24 hr.	57	39	41
Lee	86	5 min.	45	41	48
		2 hr.	38	48	56
		6 hr.	31	55	64
		24 hr.	31	55	64

TABLE 10.—EFFECT OF IRRADIATION ON ADSORPTION OF HEMAGGLUTININ

Strain of irradiated virus	Time of irradiation (min.)	Hemaggl. (units injected)	Hemaggl. (units recovered)	Hemaggl. (units adsorbed)	% adsorbed
PR8	0	207	68	139	67
	3	198	118	80	40
	10	184	85	99	54
	30	117	70	47	40
	60	<1			
Lee	3	120	60	60	50
	15	122	70	52	43
	30	132	52	80	61
	45	136	57	79	58
	60	95	16	79	83

The adsorption of hemagglutinin did not increase with time of incubation of the embryos. It did not matter whether the allantoic fluids of the injected embryo were collected 1 hour or 24 hours after injection of the irradiated virus. In each case comparable amounts of hemagglutinin were recovered (Table 9). In other experiments no changes in adsorption were noted up to 72 or 96 hours after injection. However, when the allantoic fluids were collected after 96 hours the results became increasingly more variable on account of the changes occurring in the amount and composition of the allantoic fluids at that stage rendering hemagglutination tests unreliable or impossible. Control tests showed that dilution of virus in normal

15 minutes or longer under the experimental conditions employed the adsorption of the virus on to chicken red cells[16] and on to the cells of the allantoic sac remained unchanged. In other words, as long as hemagglutination remained intact the virus was adsorbed whether it had been irradiated for a few minutes or for 1 hour or longer (Table 10).

The question whether adsorption of 1 virus, for instance influenza Type A, prevents adsorption of additional virus of the heterologous Type B has been studied in a few experiments. Ten day old chick embryos were divided into groups of 10 each. The first and second groups received irradiated PR8 virus at time 0. The second was injected again 1 hour later

together with the third group with irradiated Lee virus. The allantoic fluids of all eggs were harvested after further incubation for 1 hour, their volume measured and the content of hemagglutinin determined. The units of hemagglutinins adsorbed in the first group subtracted from the figure found for the second group represents, presumably, the units of hemagglutinin additionally adsorbed and this result should be compared with the result obtained in the third group. The allantoic fluids employed in these experiments showed marked capacities of interference with the propagation of active virus as determined in preliminary experiments. Table 11 shows the results obtained in an experiment of this kind involving 3 different preparations of PR8 virus and 1 of Lee. As can be seen, the adsorption of the Lee virus was of the same order of magnitude when injected into normal eggs or into eggs previously injected with PR8 virus. These data indicate that additional adsorption can take place on to host cells which had been previously rendered resistant to infection by the primary injection of irradiated virus. In other words, interference does not prevent adsorption. In other experiments when the primary adsorption was more marked, *i. e.*, after the initial injection of larger amounts of hemagglutinin, the evidence for adsorption of the secondarily injected hemagglutinins was negative or doubtful, an observation which suggests that a saturation point may be reached beyond which no further adsorption occurs.

TABLE 11.—ADSORPTION OF VIRUS BY CELLS PREVIOUSLY EXPOSED TO HEMAGGLUTININS OF THE HETEROLOGOUS TYPE

1st injection (strain)	2nd injection (strain)	Total hemaggl. (units injected)	Hemaggl. (units recovered)	Hemaggl. (units adsorbed)	% adsorbed	Additional hemaggl. (units adsorbed)
PR8 No. 1	..	198	118	80	40	
PR8 No. 1	Lee	198 + 86	151	133	47	53
PR8 No. 2	..	117	70	47	40	
PR8 No. 2	Lee	117 + 86	113	90	44	43
PR8 No. 3	..	184	85	99	54	
PR8 No. 3	Lee	184 + 86	134	136	50	37
	Lee	86	35	51	59	

Discussion. The data presented on interference between inactivated influenza and the propagation of the active homologous or heterologous agents in the chick embryo have added to the understanding of the interference phenomenon. The following facts appear to be established: (1) It is the inactivated virus particle which causes interference and not some product resulting from the host-virus interaction. All attempts to separate physically the virus activity from the interfering capacity have failed.[14] However, the interfering property may be based on a different grouping within the virus particle since its resistance to ultraviolet light differs from that of other properties of the virus.[16] (2) Only the host cells directly in contact with the interfering agent are resistant to infection with the active virus, whereas the remainder of the embryonic tissues, if susceptible to infection, remains so. This fact is in agreement with the observations of Kunkel,[21] made in the study of interference in virus infections of plants. (3) Resistance is induced in host cells rather rapidly within a few minutes or even seconds. (4) The host cells treated with irradiated virus remain resistant to infection with active virus for extended periods of time, *i. e.*, for at least 6 days, representing the longest interval which could be studied under the conditions of these experiments. (5) The interfering agent is not without measurable effects on the host cell activities. In the extreme case further growth of the allantoic sac is largely in-

hibited, which, in turn, slows down the embryonic development. (6) Adsorption of active or inactivated virus particles on to the cells of the allantoic sac does not lead in itself to interference, since adsorption is maintained on prolonged irradiation of the virus, whereas the interfering capacity is destroyed after much shorter exposure to ultraviolet light.[16] It is obvious then, that in addition to adsorption, a further step is required to induce interference. Furthermore, after adsorption of irradiated hemagglutinins of 1 type of virus, additional virus particles of the homologous or heterologous type may be adsorbed on to the host tissues, which had been made resistant to infection by the primarily injected agent.

These various observations are in striking agreement with and extend similar data collected by a number of authors in the study of bacterial viruses in their relation to sensitive bacteria. Thus, interference between inactivated and active bacterial viruses has been reported by Luria and Delbrück.[23] The quantitative aspects indicated strongly that the interfering agent was identical with the irradiated virus. Prolonged irradiation by ultraviolet light destroyed the interfering capacity. It was shown that the host cells ceased to multiply upon contact with the irradiated virus.[1,5,23] The inhibition of host cell multiplication occurred within a few seconds after contact of the host cells with the virus.[3] Adsorption of virus on to host cells was not prevented, although they had been rendered resistant to infection by previous contact with homologous or heterologous, active or inactivated virus.[8,23] Finally, Cohen and Anderson[5] found that the oxygen consumption of infected bacteria, or organisms treated with irradiated virus was similar to that of the normal host cells. Preliminary tests conducted by the present authors in collaboration with Dr. Cohen have shown that the oxygen consumption of the chorioallantoic membrane treated with irradiated influenza virus and showing marked reduction in growth was not different from that of normal tissue.

These comparisons show the essential similarity in the activities of 2 widely separated viral agents. Experimentation with the bacterial viruses has the advantage over the influenza host-virus system that one may study the effect of known numbers of virus particles on a known number of bacterial host cells. Thus, it could be demonstrated that 1 single virus particle *per bacterium* altered the host cell in such a way that other virus particles of the homologous or heterologous strain were excluded and further multiplication of the host was prevented.[8,23] It has not been possible as yet to obtain similarly quantitative data in the study of the activity of the influenza viruses in the allantoic sac of the chick embryo. There is no definite proof of the number of virus particles required for the infection of the chick embryo. However, data have been presented that one 50% infectivity dose of a preparation of purified influenza virus corresponded to about 10 virus particles[9] and the chances are that some of these had been inactivated during the process of purification. There is, on the other hand, no information available as yet on the number of susceptible host cells lining the allantoic sac. Further studies will be directed toward solution of this problem.

In the light of these data a number of hypotheses previously suggested by various authors for the explanation of the interference phenomenon can be regarded as invalidated at least for cases where inactivated virus produces rapid changes as recorded in the present paper. Among these one may list: (1) the prevention of spread of the secondarily injected virus by the presence of inflammatory reactions caused by the first; (2) antiviral activity of one virus for another, or the development of antiviral substance in the infected tissues as a result of host virus interaction; (3) the exhaustion by the primarily injected virus of substrates essential for the propagation of the second agent; and (4) a blockade of cell receptors to which the

virus becomes attached prior to entrance into the cells.

There remain 2 hypotheses, both of which can account for some of the observed phenomena. The first assumes the blockade of a "key enzyme" necessary for the propagation both of the virus and the host cell.[8] This hypothesis has been abandoned by one of its authors recently in favor of the second. This hypothesis[7] proposes that changes are produced in the host cell surface by the penetration of 1 virus particle into the interior of the cell which then renders the cell wall impenetrable to other viruses in analogy to the ovum-sperm relationship. However, this comparison certainly is not in agreement with one of the observed facts, the discontinued multiplication of the host. Whereas the hypothesis of the key enzyme can explain interference as well as inhibition of host propagation, the "penetration hypothesis" may account for interference only. It should be emphasized, however, that it has not been definitely proven that inhibition of propagation of the host cell and the interference phenomenon are part of the same mechanism. Although the 2 types of viruses thus far studied in this respect impaired host cell multiplication, other virus infections have resulted in hyperplasia of the host tissues. Whether this stimulation of growth is based on a direct effect of virus or indirectly accomplished by the release of some growth-promoting substance from damaged cells has not been settled as yet. On the other hand, prevention of host cell multiplication by means other than treatment with irradiated virus does not necessarily lead to suppression of virus growth. Spizizen[25] showed that certain glycin compounds were bacteriostatic but did not prevent virus production, and Anderson[2] showed that bacteria irradiated by ultraviolet light, although no longer multiplying, supported virus propagation. Other substances such as 5 methyl tryptophane prevented both bacterial and viral multiplication which, however, was a reversible reaction in contrast to the true interference phenomenon.[6] It is obvious that these problems require further study.

Summary. Further studies are reported on the interference phenomenon observed to occur between influenza viruses inactivated by ultraviolet irradiation and the active agents in the allantoic sac of the chick embryo. They are concerned particularly with the effects of the interfering agent on the host cells.

It has been shown that only cells directly in contact with the irradiated virus become resistant to infection with the active agents. Resistance is induced within less than 1 minute and lasts for at least 6 days. The allantoic sac, in addition, may show impairment of its development, which, in turn affects the growth of the embryo. Host cells rendered resistant by contact with irradiated virus of 1 type may still adsorb additional virus of the homologous or heterologous types. Virus which has lost its interfering capacity by prolonged exposure to ultraviolet light is still adsorbed on to the host cells as long as the hemagglutinating activity remains intact.

These various data are discussed in regard to possible explanations for the interference phenomenon. The striking similarities between the influenza viruses and certain bacterial viruses in their host cell relationships are pointed out.

REFERENCES

1. Anderson, T. F.: J. Cell. and Comp. Physiol., **25**, 1, 1945.
2. Anderson, T. F.: J. Bact., **47**, 113, 1944.
3. Andrewes, C. H., and Elford, W. J.: Brit. J. Exp. Path., **13**, 13, 1932.
4. Burnet, F. M., Beveridge, W. I. B., McEwin, T., and Boake, W. C.: Australian J. Exp. Biol. and Med. Sci., **23**, 177, 1945.
5. Cohen, S. S., and Anderson, T. F.: J. Exp. Med., **84**, 511, 1946.
6. Cohen, S. S., and Anderson, T. F.: J. Exp. Med., **84**, 525, 1946.
7. Delbrück, M.: J. Bact., **50**, 151, 1945.
8. Delbrück, M., and Luria, S. E.: Arch. Biochem., **1**, 111, 1942.

9. FRIEDEWALD, W. F., and PICKELS, E. G.: J. Exp. Med., **79**, 301, 1944.
10. HENLE, W., and HENLE, G.: Science, **98**, 87, 1943.
11. HENLE, W., and HENLE, G.: AM. J. MED. SCI., **207**, 705, 1944.
12. HENLE, W., and HENLE, G.: AM. J. MED. SCI., **207**, 717, 1944.
13. HENLE, W., and HENLE, G.: AM. J. MED. SCI., **210**, 362, 1945.
14. HENLE, G., and HENLE, W.: AM. J. MED. SCI., **210**, 369, 1945.
15. HENLE, G., and HENLE, W.: Unpublished data.
16. HENLE, W., and HENLE, G.: J. Exp. Med., **85**, 347, 1947.
17. HENLE, W., HENLE, G., STOKES, J., JR., and MARIS, E. P.: J. Immunol., **52**, 145, 1946.
18. HIRST, G. K.: J. Exp. Med., **76**, 195, 1942.
19. HIRST, G. K.: J. Exp. Med., **78**, 99, 1943.
20. HIRST, G. K., and PICKELS, E. G.: J. Immunol., **45**, 273, 1942.
21. KUNKEL, L. O.: Phytopathology, **24**, 437, 1934.
22. LEE, H. F., STAVITSKY, A. B., and LEE, M. P.: Proc. Soc. Exp. Biol. and Med., **61**, 143, 1946.
23. LURIA, S. E., and DELBRÜCK, M.: Arch. Biochem., **1**, 207, 1942.
24. MILLER, G. L., and STANLEY, W. M.: J. Exp. Med., **79**, 173, 1944.
25. SPIZIZEN, J.: J. Infect. Dis., **73**, 212, 1943.
26. ZIEGLER, J. E., JR., LAVIN, G. I., and HORSFALL, F. L., JR.: J. Exp. Med., **79**, 379, 1944.

COLD HEMAGGLUTININS IN SICKLE CELL ANEMIA

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Significant titers of cold hemagglutinins were found by Finland *et al.* in 4 cases of acquired hemolytic anemia.[1] Young,[5] in a survey of a large group of individuals for cold hemagglutinins, observed in 3 cases of sickle cell anemia titers of 1:256, 1:4 and 1:4. Wagley[3] has found high titers of cold hemagglutinins in a case of congenital hemolytic icterus and 2 cases of paroxysmal (cold) hemoglobinuria. The purpose of this study is to report the observations on cold hemagglutinins in a large number of cases of sickle cell anemia.

Materials and Methods. The method used for titering cold hemagglutinins was that described by Finland *et al.*[1] with variations suggested by Stats and Wassermann.[2] All samples of blood were drawn under sterile conditions at room temperature. Approximately 10 cc. of blood from the patient were placed in a dry, sterile, plugged centrifuge tube and allowed to clot. Several cc. of blood were also placed in a small bottle with 0.5 cc. of an ammonium and potassium oxalate mixture to be used for the determination of the hemoglobin, hematocrit and icterus index. The clotted blood was centrifuged at room temperature and the serum withdrawn by sterile pipette into a sterile, dry tube. Serial dilutions of the serum were made with 0.9% NaCl from 1:10 to 1:2560. Each tube contained 0.5 cc. of the dilution.

Group O cells were washed with 0.9% NaCl 3 times and resuspended in a 0.2% concentration. To each tube containing 0.5 cc. of diluted serum was added 0.5 cc. of the 0.2% erythrocyte suspension. The tubes were shaken thoroughly, packed in cracked ice and water and stored for 20 to 24 hours in a refrigerator to assure a temperature of circum 4° C. At the end of that time readings were made.

The readings were made against a well-illuminated white background. The tubes were shaken gently 3 to 5 times and read in an arbitrary grade of 4+ to 1+ or negative. A 4+ represented one solid clump of cells remaining after shaking and 1+ the least amount of agglutination grossly detectable to the naked eye. After reading, the tubes were allowed to stand at room temperature for several hours and then re-read. Only tubes in which the agglutination had disappeared were accepted as having been positive. By this technique, agglutination in a titer of 1:40 has been considered significant.

The hemoglobin determinations were made by the Fisher Electrohemanometer or the Hellige-Wintrobe method.[4] The hematocrit and icterus index determinations were made by standard techniques.

The amount of sickling was determined in each case by the use of wet preparations. These were made by placing a small drop of freshly drawn venous blood upon a clean, dry slide and covering with a dry cover-slip. The preparation was immediately ringed with petrolatum. The amount of sickling was observed immediately following preparation and at 24 hours by counting the number of sickle cells per 500 red blood cells and expressing the results in percentage.

All patients were questioned as to signs and symptoms of recent upper respiratory infection and the data recorded in Table 1. An attempt was made to correlate cold hemagglutinin titer with the degree of sickling, hemoglobin level, hematocrit, icterus index and severity of disease,† as shown in Table 1.

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† "Severity of disease" is a clinical impression based on the number, frequency and duration of crises, severity of pain and elevation of temperature during the crises, hematologic picture, necessity for transfusion, degree of activity tolerated between crises and presence or absence of leg ulcers.

The control series consisted of 30 colored patients without hemolytic anemia, sicklemia, or other blood dyscrasias, negative serologic test for syphilis and without a history of recent upper respiratory infection.

prior to and during crisis. No significant change in the titer of cold hemagglutinin (1:320) or other laboratory studies was observed. Another patient (No. 22) was

TABLE 1.—ANALYSIS OF DATA IN 30 CASES OF SICKLE CELL ANEMIA

	Titer						Sickling (%)							
	1:20	1:40	1:80	1:160	1:320	1:640	Immediate	After 24 hrs.	Hemoglobin	Hematocrit	Icterus index	Most recent URI before test done	Severity of disease	Most recent crisis before test done
1	2	1	1	..	..	..	2	55	7.2	22.4	9.0	None	Mod. severe	2 wks.
2	1	..	..	..	..	..	8	55	10.3	32.0	10.0	None	Severe	3 mos.
3	1	1	..	..	..	..	1	30	8.3	25.0	35.0	None	Mod. severe	3 mos.
4	4	3	3	2	1	..	9	95	10.0	20.6	23.0	None	Mod. severe	13 mos.
5	2	1	1	..	..	..	49	95	7.2	29.2	50.0	None	Severe	2 mos.
6	4	4	4	3	1	..	15	85	7.6	30.2	10.0	None	Severe	In crisis
7	1	1	..	..	..	..	52	80	6.2	16.0	20.0	None	Severe	5 mos.
8	1	..	..	..	..	..	32	75	5.8	18.5	35.0	3 wks.	Mild	6 mos.
9	1	1	..	..	..	..	30	70	6.4	19.8	30.0	None	Severe	5 mos.
10	1	1	1	..	..	..	16	70	5.5	23.2	25.0	None	Mild	8 mos.
11	1	1	..	..	..	..	36	75	6.6	22.0	20.0	None	Severe	5½ mos.
12	1	..	..	..	..	..	9	50	9.1	29.0	7.5	None	Mild	None
13	2	1	1	..	..	..	10	55	7.9	27.1	7.5	None	Mild	In crisis
14	1	1	1	..	..	..	0	2	9.1	31.5	7.5	None	Mod. severe	5 mos.
15	..	..	..	..	..	..	25	50	7.0	23.5	18.0	2 wks.	Severe	3½ mos.
16	..	..	..	..	..	..	5	30	10.1	30.0	10.0	1 wk.	No data available	
17	..	..	..	..	..	..	25	100	6.3	21.0	7.5	None	Mod. severe	19 mos.
18	..	..	..	..	..	..	25	90	6.6	23.0	5.0	None	Mod. severe	2 wks.
19	..	..	..	..	..	..	35	100	5.9	20.0	10.0	None	No data available	
20	1	1	..	..	..	..	50	87	6.6	21.0	35.0	None	Mod. severe	4½ mos.
21	4	4	4	2	1	..	25	61	9.1	26.0	20.0	3 wks.	Mod. severe	In crisis
22	4	4	3	1	1	1	2	10	6.3	19.5	15.0	None	Mod. severe	In crisis
23	1	1	..	..	..	..	10	38	5.5	37.0	10.0	None	Mild	In crisis
24	..	..	..	..	..	..	74	81	7.2	25.2	10.0	None	Severe	7 mos.
25	..	..	..	..	..	..	2	14	6.7	22.4	20.0	2 wks.	Mild	None
26	2	1	..	..	..	..	1	5	11.0	36.0	25.0	None	Mild	None
27	4	2	1	..	..	..	9	59	9.7	28.2	10.0	None	Mild	18 mos.
28	3	1	1	..	..	..	41	77	9.5	32.0	25.0	None	Mild	None
29	..	..	..	..	..	..	14	78	8.4	26.0	25.0	None	Mod. severe	In crisis
30	..	..	..	..	..	..	8	24	11.7	34.0	7.5	None	No data available	

Results. Table 1 shows the distribution of titers for cold hemagglutinins in 30 cases of sickle cell anemia. Eighteen cases (60%) showed a titer of 1:40 or over; 11 cases (36%) had a titer of 1:80 or higher; 4 cases (13%) had a titer of 1:160; 4 cases (13%) had a titer of 1:320; 1 case had a titer of 1:640. No correlation could be made with the degree of sickling, hemoglobin, hematocrit or icterus index. In 21 cases, the date of the last crisis could be precisely determined by hospital records. In these cases no correlation could be established between the recency or severity of the attacks and the titer of the cold hemagglutinin.

Three control cases (10%) showed a titer of cold hemagglutinin of 1:40, but none showed a titer of 1:80.

In the course of these experiments, 1 patient (No. 6) was observed 2 weeks

observed 10 months prior to crisis, at which time she showed a maximum titer of cold hemagglutinin of 1:40. During crisis a maximum titer of 1:640 was reached. There was no striking change in other laboratory findings. A third patient (No. 23) was observed to have no titer of cold hemagglutinin 2 weeks prior to crisis. During crisis a maximum titer of 1:40 was demonstrated.

Conclusion. The cold agglutinins in a series of 30 cases of sickle cell anemia were determined. Of these cases, 60% showed a titer of 1:40 or over; 36% had a titer of 1:80 or over. A significant titer could not be correlated with degree of sickling, hemoglobin level, hematocrit, icterus index, the severity of the disease or consistently with the elapse of time since the last crisis.

REFERENCES

1. Finland, M., Peterson, O. L., Allen, H. E., Samper, B. A., and Barnes, M. W. (with technical assistance of Stone, M. B.): Occurrence of Cold Isohemagglutinins in Various Conditions, J. Clin. Invest., **24**, 451, 1945.
2. Stats, D., and Wasserman, L. R.: Cold Hemagglutination: An Interpretative Review, Medicine, **22**, 363, 1943.
3. Wagley, P. F.: Unpublished data.
4. Wintrobe, M. M.: Clinical Hematology, Philadelphia, Lea & Febiger, 1946.
5. Young, L. E.: The Clinical Significance of Cold Hemagglutinins, Am. J. Med. Sci., **211**, 23, 1946.

PROPYLTHIOURACIL IN THE TREATMENT OF HYPERTHYROIDISM

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The use of propylthiouracil over a 14-month period from December, 1945, to March, 1947, has convinced us that it is decidedly superior to thiouracil in the treatment of hyperthyroidism. In our opinion propylthiouracil appears to be as effective as thiouracil in about half the dose and is much less toxic in its side effects. These findings are in agreement with those of previous reports.[1,2,3,5]

The present paper presents a brief summary of the therapeutic properties of thiouracil and discusses some of our experiences in treating 218 cases of hyperthyroidism with propylthiouracil. It deals briefly with a few of the aspects of the problem, including its safety, effectiveness, and required dosage, and contains a note regarding the concurrent use of small doses of iodine. The final place attained by any medical or surgical means for the clinical management of hyperthyroidism depends on at least one other important factor, namely its ability to produce a lasting remission or permanent cure. Insufficient time has elapsed in the use of propylthiouracil to make any sound statements on this phase of the problem. Time may reveal, however, that this point is not very important, provided the drug proves to be safe to use for long-continued periods.

Thiouracil. Thiouracil was used earlier and has been used much more widely than propylthiouracil. Before thiouracil was placed on the market it had been used in the treatment of more than 5000 patients and had been abundantly proved to be an effective means of controlling any type of hyperthyroidism with the possible exception of thyroid crisis, in which its action was too slow. Hyperthyroidism of acromegaly may also prove to be an exception.

The rate of response to thiouracil is not easily predicted. A fall in basal metabolic rate of 1% per day is seldom exceeded, while at the opposite extreme some patients fail to show any response of consequence for four or five months but later are well controlled. In general, hyperthyroidism associated with the small diffuse goiter responds quickest and that associated with the large nodular goiter slowest. The prolonged use of large doses of iodine preceding thiouracil therapy is a barrier to prompt response.

The main disadvantage of thiouracil is that toxic effects such as fever, rash, or nausea occur in 10 to 15% of the patients. Much more serious is the agranulocytosis which has been seen in 2 to 3%; death from this occurs in 0.5 to 1%. In addition, a striking enlargement of the thyroid has occurred in some patients. These effects have prevented the use of the drug in many instances. Though its effectiveness is high, the mortality rate from propylthiouracil is comparable to that of thyroidectomy in the hands of the most skillful surgeons. Thiobarbital, which is much more potent in its antithyroid action, has similar toxic effects.

The rate of permanent remission of hyperthyroidism with thiouracil therapy has not been determined. One of the best estimates is that of Williams,[6] who reported a remission rate of 50% in 100 cases. It is possible that the general tendency toward early withdrawal of the

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drug because of the risk of toxic effects may have reduced the number of permanent recurrences.

Present Usefulness of Thiouracil. One of the chief advantages of this therapy is the production of complete control of hyperthyroidism and the fact that almost all patients are able to follow their usual occupations. The complications of hyperthyroidism are controlled as well by thiouracil as by thyroidectomy.

Since thiouracil is no safer than surgery in uncomplicated moderate hyperthyroidism, and since it has not been proved to cause remissions as often as thyroidectomy, its greatest benefit is the reduction of surgical risk in selected patients. If used in severely toxic patients, those with cardiac failure, those who are weak from long-standing disease, or in those of advanced age, many will become good surgical risks. In those who remain bad risks continued therapy may be warranted. By limiting the use of thiouracil in this way the mortality of thyroid surgery has been reduced almost to the vanishing point, but the mortality rate of the drug itself remains unchanged.

PROPYLTHIOURACIL. It was evident from experience with thiouracil that a safer drug was needed, and 6-n-propylthiouracil appears to have supplied this need.

Astwood,[1] in the first 100 cases reported, found 4 instances of transient pruritus, 2 of transient headache, and 1 of transient arthralgia. One of his patients died during therapy apparently from progressive hepatic failure due to extensive cirrhosis.

Among our 218 patients, manifestations which we attributed to the toxicity or sensitivity to propylthiouracil were seen in 7 patients. Three had mild reactions: nausea, arthralgia, arthralgia and numbness. The drug was continued in all 3 of these patients. The first patient was nauseated while taking 300 mg. a day, but could take 200 mg. without discomfort. In the patients who complained of arthralgia or numbness of the forearms and hands, the symptoms disappeared while the drug was continued. No hypothyroidism or any evidence of organic nervous disease could be demonstrated in them. Propylthiouracil was discontinued in 4 patients. One patient developed a severe dermatitis simulating the exfoliative type while taking 50 to 150 mg. a day after 34 weeks of treatment. Skin testing or further trial of the medicine has been delayed in this case because of the severity of the dermatitis. The second patient complained of numbness of the extremities. This probably was a toxic effect, since no other cause was found to explain it. One showed a fall in the granulocyte count to as low as 25% on repeated trials of 75 to 150 mg. per day, so the drug was finally discontinued after 22 weeks of treatment. This case has been reported.[2] In one patient the drug was discontinued because of urticaria, and thyroidectomy was performed. One patient died at his home while taking the drug. He was 65 years old, had a nodular goiter and poor cardiovascular status. One day, without having complained more than usual, he vomited, became extremely weak, and died within a day. He had no fever. Blood counts were not made. Unfortunately, news of his death did not reach us at once. In spite of contact made with his relatives and the physician called in, no further information could be obtained, and the cause of his death was never ascertained. The meager information we received did not appear to point toward a toxic reaction, but such a possibility cannot be entirely excluded.

The final appraisal of the side effects of propylthiouracil, based on 218 separate clinical trials covering an average of 8 months per patient and using doses from 50 to 500 mg. a day, confirms to us the fact that it is a relatively non-toxic drug. To date the maximum doses used have caused no more toxic effects than have followed the small doses. There were no fatalities, 3 minor reactions which did not interrupt treatment, and 4 reactions which necessitated stopping the drug. Agranulo-

cytosis did not occur. Consequently, we have discontinued the routine practice of taking weekly white blood counts. In prescribing the drug the possibility of a toxic effect is explained to the patient, and it is suggested that a white blood count be made if a sore throat or unexplained fever occurs.

Effectiveness and Required Dose. The speed of response is an important consideration. The continuance of hyperthyroidism in a patient with complicating heart disease, diabetes, or tuberculosis may lead to an irreversible downhill course. Even without concurrent disease it is highly desirable to lower the basal metabolic rate as quickly as possible. At best the treatment covers several months, and the patient may be discouraged and lose contact with his physician if the results are too gradual. For this reason, the smaller initial doses of 150 mg. per day, as recommended in earlier publications,[2] have been replaced by doses of 300 mg. or more per day in moderately severe and in severe hyperthyroidism. Somewhat smaller doses may be sufficient for maintenance, but even this must be taken as a guarded conclusion.

All of our patients have remained ambulatory and have been treated as office patients except those who were seriously debilitated by complications such as cardiac decompensations of severe degree. Almost all others continued to work or to carry on their usual activities. The basal metabolic rate was determined at intervals of 4 weeks. A few patients received iodine in doses of 10 to 30 mg. per day in addition to propylthiouracil for comparative purposes. When the metabolic requirements were high and there was a tendency to weight loss, diets supplying as much as 4000 calories per day were prescribed and vitamin B complex was given to a few. Sedatives, such as phenobarbital, were used on doses of ½ gr. 3 times a day in many. Otherwise no other medication was given except that which was necessary to treat a complicating disease.

Eighty-seven patients who had responded with complete control of their symptoms and basal metabolic rates were used to compare the speed of effect of the drug at different dosage levels. The remainder were eliminated from this part of the study for various reasons. Some had been treated for too short a time; in some propylthiouracil was used to maintain a remission which had been effected by thiouracil, iodine, or *x*-ray; a small group failed to take the drug as prescribed, and finally in some the basal metabolic rate did not show an adequate response due to complicating factors such as asthma or poorly compensated cardiovascular disease and thus was not an accurate indication, despite control of the hyperthyroidism.

Of the 87 patients 51 had diffuse goiter, 22 had nodular goiter, and 14 had recurrent or residual hyperthyroidism following previous operation. The usual sex incidence was present. The original basal metabolic rate ranged from +19 to +79%. Complicating disease included rheumatic and arteriosclerotic heart disease, syphilis, diabetes, and pernicious anemia.

The dose of propylthiouracil varied from 50 to 400 mg. a day. Early in the study, doses of 75 or 100 mg. per day were common, but as our experience increased we gradually raised the dose to the higher levels in order to obtain a more rapid response. To study the comparative effectiveness of various doses, these cases have been arbitrarily divided into two groups: (1) those receiving less than 150 mg. per day and (2) those receiving 150 mg. or more per day.

In the 48 patients with diffuse goiter receiving 150 mg. or more a day, there was an average fall of basal metabolic rate of 2.91% per week, while in 3 patients with diffuse goiters receiving less than 150 mg. a day the fall was 2.64% per week. The most rapid response was seen in a patient having recurrent hyperthyroidism with a basal metabolic rate of +60% which fell to −11% in six weeks, an average of 11.8% per week. The dose was 200 mg. per day.

In the 15 patients with nodular goiters on the higher dose, the fall was 2.49% per week as compared with a fall of 1.74% per week in 7 patients on the smaller dose. In recurrent or residual hyperthyroidism the fall was 2.96% per week in 8 instances while it was only 1.18% per week in 6 patients receiving less than 150 mg. per day.

Table 1.—Results of Different Dosages of Propylthiouracil

	Group 1. (Less than 150 mg. per day) 16 patients	Group 2. (150 mg. or more per day) 71 patients
Average fall in B.M.R per week	1.73%	2.82%
Average original B.M.R.	23.5%	+38%
Average duration of treatment before B.M.R. returned to normal	13.6 weeks	13.6 weeks

Our data were analyzed with respect to effective dose range in another way. For this purpose an effective dose was defined as one which produced a progressive fall in basal metabolic rate of 2% or more per week to within normal range. An effective dose might take more than 3 months at this rate to produce a fall in the basal metabolic rate to within normal range, depending upon the height of the metabolic rate at the beginning of the test. If 3 months passed, however, and a rate of fall of basal metabolic rate less than 2% per week still persisted the dose employed was called ineffective. A single patient might fail to respond adequately to 150 mg. or even 200 mg. per day and yet respond well to 300 mg. or more. Thus more than one test might be made on 1 patient. In the following figures 158 such tests are represented. The results are summarized in the following table.

Table 2.—Estimated Effectiveness of Different Dosages of Propylthiouracil

Daily dose	Total No. of tests	Effectiveness	Per cent effective
150 mg. and less	50	29	58.0
200 mg. and less	111	97	87.4
300 mg. and less	158	152	96.3

Thus a dose of 300 mg. per day is seen to be ineffective, if defined in these terms, in only 3.7% of these patients. In them, doses higher than 300 mg. per day were required. We have seen little disadvantage in using 300 mg. as the initial dose or in continuing it. The basal metabolic rate in many instances drops rapidly down to +20 or +15% and slowly from that level to zero. Often, to be sure, a good response will be made on 150 mg. until the basal metabolic rate reaches +15%, and then more than 200 mg. per day is necessary to relieve the remaining signs of hyperthyroidism. In a few patients the dose had to be raised even to 400 mg. or more to obtain a normal metabolic rate. Four hundred milligrams or more per day have been used at some time during the treatment of 25 patients.

Frank myxedema occurred twice, and definite symptoms of hypothyroidism appeared in 5 other patients. Desiccated thyroid was given to control this complication, and the propylthiouracil was continued. We wished to maintain the depressive effect on the goiter in the hope of increasing the chance of securing a permanent remission. Only 2 patients showed significant enlargement of the thyroid. One of these was a young woman who was pregnant, the other a boy 17 years of age. We have not been impressed by the presence of a lethargy or drowsiness which might be regarded as an effect of the drug apart from the hyperthyroidism.

We interpret these data to mean that 300 mg. per day of propylthiouracil is near the ideal dose to be used in active treatment of hyperthyroidism. Although some patients respond to smaller doses, a dose of 300 mg. or more a day has been necessary in 34% of the total number of patients in order to lower the basal metabolic rate to normal range. If toxic results on doses of 300 mg. or more per day are shown to be more frequent in further studies, the routine use of this initial dose will be modified. If such circumstances arise,

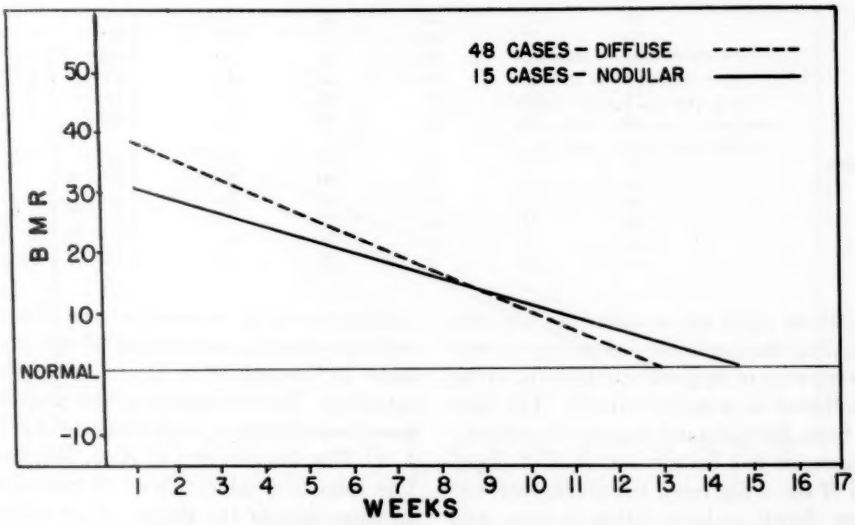
200 mg. may be tried and the dose increased if an effective response fails to occur. In this way time would be lost, but the minimum amount of the drug would be used.

With regard to speed of control a few general statements may be made. Hyperthyroidism in association with small diffuse goiters in cases in which no previous iodine has been given, react the most rapidly to propylthiouracil. These promise for this reason to be among those which will respond eventually with a permanent medical cure. Instances have been seen, however, especially in young people, in which increased dosage of the drug is associated with only partial control, appearing to indicate a natural ingravescence of the disease. In some of these the thyroid continues to enlarge during treatment. These patients appear to us to be of the same type as those in whom more than one recurrence may occur following operation. Such resistance to treatment certainly does not encourage hope for a permanent medical cure.

Hyperthyroidism of long standing has, in most instances in our hands, been more resistant to therapy. The goiter is usually larger under such circumstances, and perhaps the mass of reacting thyroid tissue present is a factor of some importance. We are inclined to believe that it is.

Large adenomatous goiters respond more slowly in some instances. If groups of cases are compared, however, it is interesting to note that there is only a small average difference. The impression that the opposite is the case apparently arises chiefly from the fact that there are some very rapid responses among the patients with diffuse goiter and a few very

AVERAGE RESPONSE TO PROPYL THIOURACIL IN DIFFUSE AND NODULAR GOITER



Average fall in B M R per week --- Diffuse goiter 2.66% - Nodular goiter 2.37%.
The average response differs little in diffuse and nodular goiters.
The very rapid responses tend to occur in the diffuse.
The very slow responses of many months occur chiefly in large nodular goiters.

CHART 1

slow responses among those with nodular goiter. Chart 1, showing average response in diffuse and in nodular goiter, illustrates this point.

A woman with acromegaly was the only patient with hypermetabolism which we judged to be at least partly of thyroid origin who has not responded at all to propylthiouracil. She has had as much as 600 mg. per day for more than 9 months.

Table 3.—Degree of Exophthalmos Before and After Treatment

	Degree of exophthalmos				Duration of treatment weeks	B.M.R	
	Before treatment		After treatment				
	O.D.	*O.S.*	*O.D.*	*O.S.*		First	Last
Men	21	22	22	22	37	+44	−18
	30	27	30	27	46	+40	− 5
	21	21	26	24	37	+62	− 9
	23	23	22	22	43	+24	+15
	21	21	25	23	28	+15	− 1
	19	19	27	28	14	+53	+ 5
Women	19	19	19	19	48	+29	+11
	21	21	20	20	57	+28	+ 9
	25	25	25	24	59	+16	− 5
	26	24	28	27	48	+61	+10
	26	25	26	26	37	+28	+ 0
	21	20	21	20	27	+52	− 5

Little of value can be said from our data regarding the probable frequency of permanent cure of hyperthyroidism following withdrawal of propylthiouracil. The drug has been discontinued in only 17 patients. Recurrence has been noted in 6 of these. In 4 of the 6 the basal metabolic rate was never shown to have fallen to zero, and in 1 of these the drug was stopped inadvertently by the patient when the basal metabolic rate was +15%. In this instance there was more accurately an ingravescence than a recurrence.

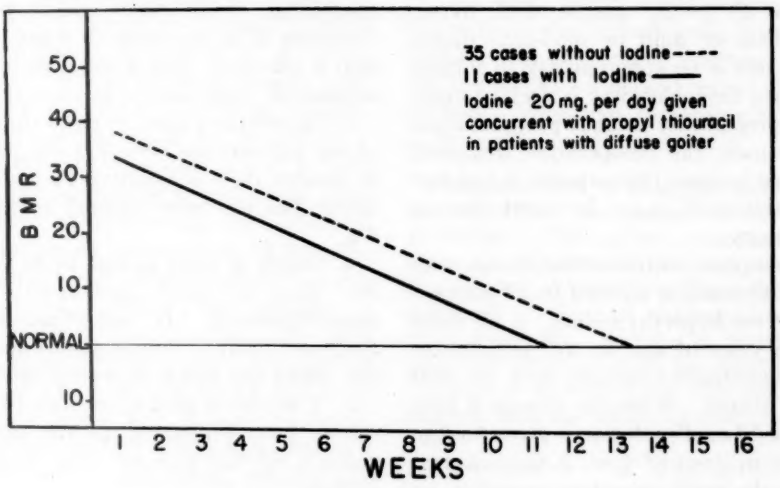
In 2 patients, after treatment for 36 and 51 weeks, respectively, there was a recurrence in 12 weeks and in 4 weeks after cessation of medication, the basal metabolic rate to cessation being −9 and −15%, respectively. In 11 other patients remission continued for periods ranging from 1 to 30 weeks in 10 and up to 14 months in 1.

Exophthalmos. Exophthalmos appears to behave during treatment with propylthiouracil in much the same way as it does after thyroidectomy; namely, in the majority it remains approximately the same or there is a slight increase, in a few there is an increase which occasionally may be extreme. The rate of increase in exophthalmos and its degree of severity appear to bear little or no relationship to the activity of the thyroid gland. In most of our patients an attempt has been made to follow the course of exophthalmos and associated eye signs by repeated estimation of the severity of the changes and by actual measurement of the proptosis by means of a Hertel exophthalmometer. Measurements of the proptosis have been made in each instance by Dr. A. D. Ruedemann or Dr. R. J. Kennedy. The following table (Table 3) represents measurement of the degree of exophthalmos in millimeters in a group of 12 patients in whom 3 or more measurements were made over a period varying between 14 and 59 weeks. The A.P. position of the globe is given at the beginning and at the end of this course of therapy and is stated in millimeters, and concurrent fall in basal metabolic rate is shown.

Iodine and Propylthiouracil Given Concurrently. Early in the use of thiouracil desiccated thyroid was used in doses as high as 2 gr. per day in the 3 patients in whom it was tried but failed to eliminate the thrill and bruit from a vascular goiter over a period of a month. Iodine, however, in doses of 10 mg. per day did so in less than a week. It became a matter of interest to know whether such manage-

ment hindered or helped the antithyroid effect as a whole or interfered with propylthiouracil later. A group of patients who had diffuse goiters of approximately the same size was chosen. Some of these were given iodine in doses of 10 to 30 mg. per day concurrently with propylthiouracil. The remainder were given no iodine. There was no significant difference in their rate of response. The following chart (Chart 2) depicts the average rate of fall

COMPARISON OF AVERAGE RESPONSE TO PROPYL THIOURACIL WITHOUT IODINE AND WITH IODINE



Average fall BMR per week—without iodine 2.95% -- with iodine 3.07%.
Iodine 10 mg. per day quickly eradicates the thrill and bruit which may appear.
In these doses the effectiveness of propyl thiouracil is not hindered.
Pretreatment with iodine should not be suddenly stopped.

CHART 2

of the basal metabolism in the two groups. For this reason it has become our practice to give iodine in doses of 10 to 30 mg. per day in cases of Graves' disease to prevent these signs of vascularity within the gland. It is possible that larger doses of iodine might be used concurrently with propylthiouracil treatment to produce a more rapid response without interference with the final propylthiouracil effect. In view of experimental work bearing on this phase of the problem it must be approached with caution since it has been shown that the thyroid glands of thiourea-treated rats fail to take up iodine in the normal manner,[4] and also because experience has shown that thiouracil effect may be slowed by pre-treatment with large doses of iodine.

The proper rôle of propylthiouracil in the management of hyperthyroidism has not been settled and cannot well be settled until the matter of the control of increscence of the disease during therapy and especially the production of lasting remissions after cessation of therapy has been adequately established.

At present our procedure is as follows, the patients having been graded into the following 4 classes:

1. In patients with small goiters and relatively mild hyperthyroidism an attempt at a medical cure is made. The aim here is to control the symptoms completely and if possible to hold the basal metabolic rate at or below zero for 8 or more months. Exception to this manage-

ment may arise for various reasons including: (1) the patient's preference for the relative certainty of a surgical cure, (2) lack of intelligence and coöperation of the patient, (3) interference of toxic effects, (4) existence of a solitary adenoma and indication for surgical removal, (5) presence of pregnancy with consideration of surgery as the more prudent course until safety of the drug to the infant is fully established.

If after adequate trial and withdrawal of the drug recrudescence is evident, a choice between its continued use and surgery must be made.

2. In all young people with hyperthyroidism of mild or moderate degree when there is no apparent risk to surgery and when thyroidectomy is decided upon, iodine preparation without propylthiouracil is usually the preoperative treatment of choice because the response is quicker. Propylthiouracil may be used but is unnecessary.

3. Complete control of the disease with propylthiouracil is advised in all patients with severe hyperthyroidism, in all those over 45 years of age, as well as in those with complicating factors such as poor cardiac status. When the disease is thus controlled Lugol's solution is given for 2 to 3 weeks in doses of 1 cc. 3 times a day. During the week preceding operation no propylthiouracil is given and thyroidectomy is performed.

4. In patients whose hyperthyroidism is complicated by extreme old age, by cardiac or other complications which will prevent their ever becoming good surgical risks even after the elimination of hyperthyroidism, propylthiouracil may be continued indefinitely. To this group may also be added those patients with postoperative recurrence of hyperthyroidism because in them the increased morbidity from nerve or parathyroid gland injuries warrants withholding further operation unless it becomes imperative.

Conclusions. 1. Propylthiouracil is a safe drug. In 218 patients treated for an average of 8 months and in some as long as 14 months, no cases of agranulocytosis were seen. Three minor reactions occurred, and 4 other reactions required the cessation of treatment.

2. It is effective in controlling hyperthyroidism of all types except possible instances of acute crisis in which its action is too slow. The hyperthyroidism of acromegaly may also be an exception.

3. An effective dose in more than 95% of our patients has been 300 mg. per day. A smaller dose is effective in many. A larger dose has been required in less than 4%.

4. Iodine in doses as high as 30 mg. per day may be used concurrently with propylthiouracil. It will eliminate the thrill and bruit in diffuse glands and does not hinder the action of propylthiouracil.

5. A tentative plan is presented for the use of propylthiouracil to the best advantage of the patient with hyperthyroidism.

6. The probable frequency of permanent remissions of hyperthyroidism following the use of propylthiouracil cannot yet be properly estimated. At present long-continued use of the drug appears warranted in some patients and may later appear feasible and perhaps desirable in many.

REFERENCES

1. Astwood, E. B., and VanderLaan, W. P.: Treatment of Hyperthyroidism With Propylthiouracil, Ann. Int. Med., **25**, 813, 1946.

2. McCullagh, E. P., Ryan, E. J., and Schneider, Robert: Propylthiouracil in Treatment of Hyperthyroidism, Cleveland Clinic. Quart., **13**, 232, 1946.

3. McGavack, T. H., Gerl, A. H., Vogel, Mildred, and Schutzer, Seymour: Clinical Comparison of Effectiveness of 6-n-propylthiouracil and s-thiouracil as Anti-thyrotoxic Agents, Am. J. Med., **2**, 144, 1947.

4. Rawson, R. W., Tannheimer, J. F., and Peacock, W.: Uptake of Radioactive Iodine by Thyroids of Rats Made Goiterous by Potassium Thiocyanate and by Thiouracil, Endocrinology, **34**, 245, 1944.

5. Reveno, W. S.: Propylthiouracil in Thyrotoxicosis; Observations on Fifty-four Patients, J. Am. Med. Assn., **133**, 1190, 1947.

6. Williams, R. H.: Thiouracil Treatment of Thyrotoxicosis; Results of Prolonged Treatment, J. Clin. Endocrinol., **6**, 1, 1946; also Williams, R. H., Clute, H. M., Anglem, T. J., and Kenney, F. R.: Thiouracil Treatment of Thyrotoxicosis; Toxic Reactions, J. Clin. Endocrinol., **6**, 23, 1946.

USE OF PYRIBENZAMINE IN PREVENTION OF HISTAMINE-INDUCED GASTRIC ACIDITY AND HEADACHE AND IN TREATMENT OF HYPERSENSITIVENESS TO COLD*

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PYRIBENZAMINE† is one of a series of compounds the ability of which to antagonize the effects of histamine recently has been investigated by Mayer, Huttrer and Scholz.[5] The chemical name is N′-pyridyl-N′-benzyl-N-dimethyl-ethylene diamine hydrochloride. The drug is a white, crystalline, stable non-hygroscopic compound, soluble in water at room temperature to the extent of 130 gm. per 100 cc.

According to the aforenamed investigators, pyribenzamine in very small doses is capable of preventing the action of histamine on an isolated strip of intestine of the guinea pig. It also has anti-acetylcholine activity, but for such purpose, a dose 100 times that necessary for antihistamine action is required. The authors named also have demonstrated that pyribenzamine is effective in delaying occurrence of the asthma which exposure to a histamine-aerosol-air stream usually produces in guinea pigs. Under ordinary circumstances, asthma appears in 35 to 90 seconds and convulsions in 1½ to 3½ minutes after exposure to histamine-aerosol-air stream. The convulsions are delayed from 2 to 6 hours if pyribenzamine is administered subcutaneously in the amount of 0.1 mg. per kilo. of body weight 15 minutes prior to exposure. According to Mayer,[4] the drug also protects dogs and guinea pigs against shock doses of anaphylactic substances to which the animals have been sensitized.

The mechanism of the action of pyribenzamine and other antihistamine compounds is unknown.

FAILURE OF PYRIBENZAMINE USUALLY TO PREVENT THE INCREASE IN GASTRIC ACIDITY INDUCED BY HISTAMINE. Fourteen persons were used in the study. Twenty-seven gastric analyses were done. The patients studied had multiple sclerosis, Ménière's disease, nerve deafness or tension headaches. A normal person also was studied. None had symptoms referable to the gastro-intestinal tract.

Procedure. The volume, free acidity and total acidity of the gastric content were determined. The subject was then given 0.05 mg. of histamine base intravenously. The solution of histamine diphosphate used contained 0.1 mg. of histamine base per cubic centimeter. Samples of gastric juice were then obtained every ten minutes over a period of approximately one and a half hours or until the values returned to fasting level.

Pyribenzamine was then given for a specified period of time. Dosage of the drug varied from 50 mg. twice daily to 100 mg. four times daily. The period over which it was administered varied from one to eight days, the last dose being given the day of the second test in most instances. Fasting levels of volume and free and total acidity were again determined. Then intravenous injection of 0.05 mg. of histamine base was given for the second time and analysis of gastric content was repeated in the same manner as previously described.

Results. Gastric acidity was increased after the 1st injection of histamine in 12 of the 14 cases. In 2 cases histamine failed to cause increased acidity. Of the 12 cases in which increased acidity was

* Abridgment of a portion of thesis to be submitted by Dr. Perry to the Graduate School of the University of Minnesota, in partial fulfillment of the requirements for the degree of Master of Science in Medicine.

† Ciba Pharmaceutical Products, Inc.

found after the 1st injection of histamine, in all but one acidity was increased after a 2nd injection of histamine given subsequent to the use of pyribenzamine. In 2 cases (one illustrated in Fig. 1) a less pronounced rise occurred after use of pyribenzamine, in 4 cases essentially the same results as in the initial observations were secured and in 4 cases (one illustrated in Fig. 2) an increase in acidity was found. The foregoing determinations were not made on one person after use of pyribenzamine because the occurrence of severe side reactions, including dizziness and numbness of the hands, necessitated discontinuance of administration of the drug. Of the 2 persons whose acidity failed to increase after the 1st injection of histamine, 1 responded and 1 failed to respond to injection of histamine after the

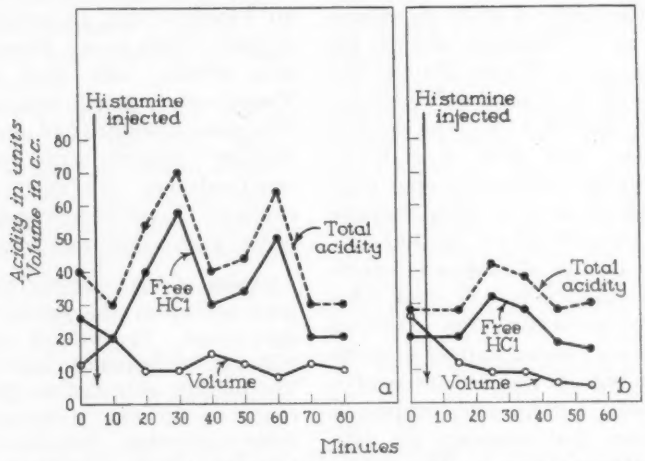

FIG. 1.—Alterations in volume and acidity of gastric contents in a case of nerve deafness: (*a*) after intravenous administration of 0.05 mg. of histamine base; (*b*) after treatment with 1600 mg. of pyribenzamine followed by intravenous administration of 0.05 mg. of histamine base.

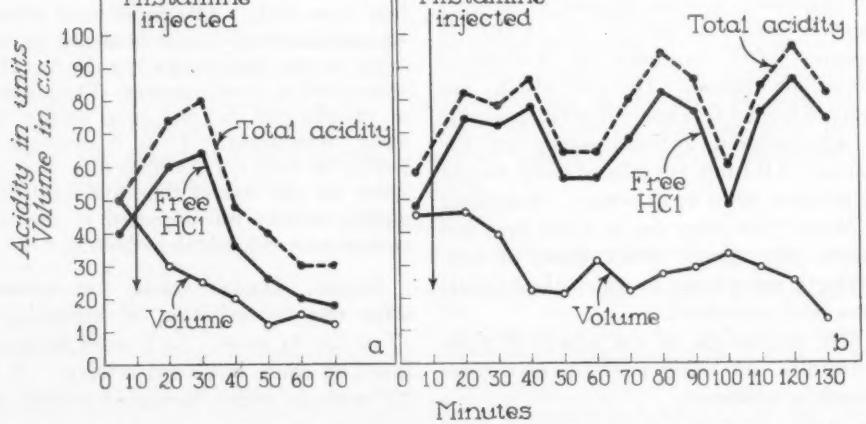

FIG. 2.—Alterations in volume and acidity of gastric contents in a case of multiple sclerosis: (*a*) after intravenous administration of 0.05 mg. of histamine base; (*b*) after treatment with 1000 mg. of pyribenzamine followed by intravenous administration of 0.05 mg. of histamine base.

use of pyribenzamine. Average values for the preceding determinations for 13 persons are shown in Table 1.

Comment. We cannot explain the foregoing results. McElin and one of us[3] (Horton) previously found in similar tests that benadryl similarly did not have a consistent effect in preventing an increase of gastric acidity after administration of histamine. Recently it has also been demonstrated[7] that neither benadryl nor pyribenzamine has the ability to antagonize histamine-stimulated gastric secretion in the dog.

Table 1.—Failure of Pyribenzamine Usually to Nullify Histamine Effect on Gastric Secretion

	Effect of histamine only		Effect of histamine when preceded by pyribenzamine	
Gastric secretion	Before histamine	After histamine	After pyribenzamine only	After pyribenzamine plus histamine
Volume in cc.	33	..	27	..
Free acidity, units	38	56	41	64
Total acidity, units	49	69	53	76
Increase in free acidity, units	..	18	..	23

The Effect of Pyribenzamine on Histamine-induced Headache. Pickering[6] studied the effects of histamine in producing headache experimentally. Actual headache, he found, is preceded by flushing of the face, a throbbing sensation in the head and a fall in systemic blood pressure. The pressure of cerebrospinal fluid was observed by Pickering to increase markedly as the blood pressure decreased. In his work, headache usually came on in about 60 seconds after administration of histamine concomitant with return of the blood pressure to normal or above and with fall of cerebrospinal fluid pressure toward normal. Clark, Hough, and Wolff[2] made similar observations. They thought that the cause of the headache was increased dilatation of the intracranial vessels which resulted in production of painful impulses while the vessels were under normal or increased vascular tension. Schumacher, Ray and Wolff[8] in later studies concluded that extracranial and dural vessels play a minor rôle in the production of experimental histamine-induced headache, and that the 5th cranial nerve and upper cervical nerves were the afferent pathways for the conduction of pain stimuli from the intracranial vessels or surrounding structures.

Material and Procedure. Four student nurses volunteered as subjects for our observations of the effect of pyribenzamine in preventing headache induced by intravenous administration of histamine.

Determinations of basal blood pressure were obtained with the subjects supine. Then 0.5 cc. of a solution of histamine diphosphate containing 0.1 mg. of histamine base per cubic centimeter was injected intravenously. Thereafter blood pressure was determined frequently and the subject was instructed to indicate the exact time of onset of headache, its location and severity and any changes that occurred. Following cessation of headache, pyribenzamine was administered intravenously in the following amounts: in 2 instances, 25 mg. of pyribenzamine in 100 cc. of physiologic saline solution, in the 3rd instance, 25 mg. in 150 cc. and in the 4th instance, 12.5 mg. in 100 cc. were administered. Then another intravenous injection of histamine in the same amounts as previously used was given and the observation repeated.

Results. In 3 of the 4 instances, the headache caused by injection of histamine was much less intense and of shorter duration after administration of pyribenzamine than before (Fig. 3). In the 4th instance headache did not develop and a decrease in blood pressure was not observed after injection of histamine. In all 4 instances there was also a definite decrease in flushing of the face and throbbing sensation in the head when the injection of histamine followed administra-

tion of pyribenzamine. As a control study, we observed that after a subject had completely recovered from headache induced by intravenous administration of 0.5 cc. of histamine diphosphate containing 0.05 mg. histamine base a similar or even worse headache could be induced by injecting again the same amount of histamine intravenously.

The woman was seen at the clinic in August, 1941, when desensitization to histamine was started. It was estimated that at the end of 16 days the patient showed about 65% improvement as measured by the amount of swelling which occurred after immersion of the hand in water at 10° C. for 7 minutes.

When the patient was again seen at the clinic, in 1946, she complained of inability

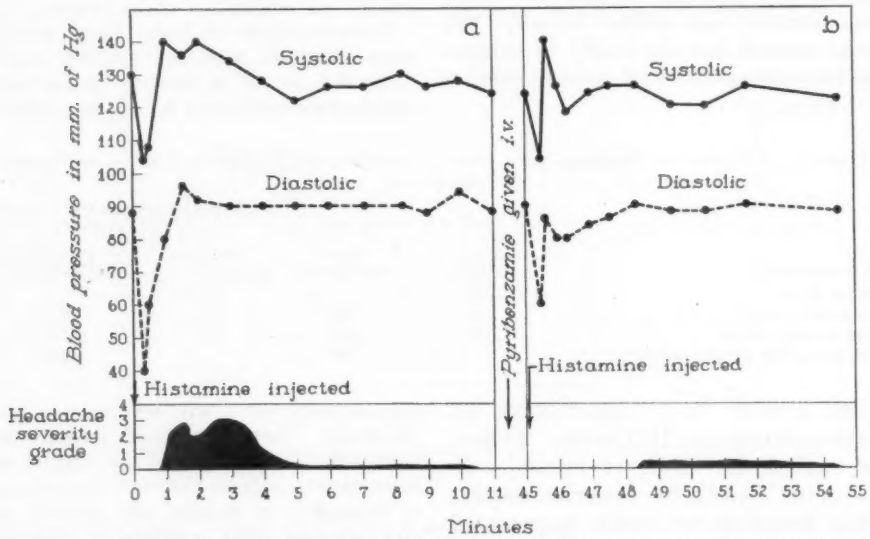

FIG. 3.—Changes in blood pressure and in severity of headache of a normal person: (*a*) induced by intravenous administration of 0.05 mg. of histamine base; (*b*) induced by intravenous administration of 0.05 mg. of histamine base after intravenous injection of 25 mg. of pyribenzamine in 100 cc. of physiologic saline solution.

Comment. Presumably pyribenzamine prevents the action of histamine on the cerebral vessels. Three of the 4 subjects experienced moderately severe side reactions consisting of dizziness, nausea, weakness and a feeling of pressure in the head.

EFFECT OF PYRIBENZAMINE IN TREATMENT OF PATIENTS WHO ARE HYPERSENSITIVE TO COLD.

Case Reports. *Case 1.* A white woman, aged 19 years, had first exhibited hypersensitivity to cold in 1941. She had gone swimming on May 30 and again on July 4. On the latter date, after she had been in the water about 2 minutes she became dizzy and fainted. When she was taken from the water, her body was red and edematous and her breathing was difficult. Some edema had persisted for about 42 hours.

to eat cold foods or to breathe cold air without occurrence of a sense of constriction in the throat. A rash would develop after exposure to extreme cold. Headache would also occur after exposure to cold.

A wheal was not produced by the application of an ice cube to the dorsum of the forearm for 3 minutes. However, the patient reported a sense of constriction in the throat and a headache after ingestion of ice water. Symptoms would appear in 2 to 3 minutes and would persist for 10 to 12 minutes. Benadryl administered in amounts of 50 mg. four times daily gave approximately 75% relief of symptoms. Pyribenzamine administered in the same dosage gave about 25% relief. Larger doses of the latter drug were tried but had to be discontinued because of the occurrence of a severe headache as a side reaction.

Case 2. A white man, aged 35 years, had had swelling of the face and hands after exposure to cold during the previous 5 years.

A wheal and a flare were produced by application of an ice cube for 3 minutes to the ventral surface of the left forearm. Immersion of the hand and forearm in water at 10° C. for 12 minutes resulted in edema of the hand, including the fingers. The edema, which extended up to the line of immersion, persisted about 7 hours. Pyribenzamine was given, 150 mg. at night and 150 mg. the following morning, after which the afore-mentioned tests were repeated. A wheal was not produced by application of an ice cube to the ventral surface of the forearm; however, edema of the hand and forearm occurred after immersion for 12 minutes in water at 10° C. The patient was then given another dose of 150 mg. of pyribenzamine. The edema in this case lasted for only an hour.

Case 3. A student nurse, 20 years of age, had urticaria after exposure to cold air. She was observed on one occasion after she had been exposed to cold air for a few minutes and urticaria had developed on the arms, forearms and thighs. The urticaria was accompanied by itching. A dose of 15 mg. of pyribenzamine in 100 cc. of physiologic saline solution was administered intravenously at the rate of 12 drops per minute. The pruritus ceased in 3 minutes and the urticarial wheals disappeared in approximately 10 minutes. The drug apparently had no effect on swelling of the lip which had appeared concomitantly with the urticaria. The patient became drowsy during administration of pyribenzamine.

She subsequently took the drug orally with prophylactic or therapeutic intent; she found that urticaria would not develop after prophylactic use of the drug and that pruritis and urticaria, once they had begun to develop, would disappear after therapeutic use of the drug. Two weeks after she had made the foregoing observations she reported that the drug seemed to have lost some of its effectiveness and that she then required 100 mg. to produce the same benefit as 50 mg. had given previously. This patient also tried the effect of benadryl and found that it took 100 mg. of benadryl to produce the same effect as 50 mg. of pyribenzamine.

Arbesman, Koepf and Lenzner,[1] in a clinical report on the use of pyribenzamine, found it effective in preventing the wheal response to application of an ice cube in cases of physical allergy to cold, as well as in enabling patients to tolerate cold without discomfort.

Comment. We should point out that in our Case 1 the manifestations were entirely subjective. In Case 2 the edema persisted for only about an hour after administration of pyribenzamine as compared with a duration of 7 hours when the drug had not been used. Urticaria, which was the manifestation in Case 3, disappeared shortly after administration of pyribenzamine. Both benadryl and pyribenzamine were used in 2 of the cases under discussion. One of the 2 patients derived greater symptomatic relief from benadryl while the other showed greater response to pyribenzamine when the drugs were given in the same dosage.

Summary and Comment. Pyribenzamine, a new antihistamine compound, has been studied with respect to its effectiveness in preventing the rise in gastric acidity induced by administration of histamine, in prevention of headache initiated by intravenous injection of histamine, and in respect to its effect on the symptoms and signs of hypersensitiveness to cold exhibited by 3 patients.

There was no consistent effect of the drug in preventing the increase of gastric acidity induced by administration of histamine. The amount of pyribenzamine used in these cases varied from 100 to 400 mg. daily given in divided doses and the drug was administered over periods varying from 1 to 8 days.

Headache induced by intravenous injection of 0.05 mg. histamine base was almost entirely prevented by intravenous administration of 12.5 to 25 mg. of pyribenzamine. Because of side reactions, we do not recommend that it be administered intravenously. We did not, however, observe any acute hypotension or other alarming reactions following intravenous use of the compound.

Three patients who had symptoms or signs of hypersensitiveness to cold were studied. Pyribenzamine was of some value in all 3 cases in reducing the severity or duration of symptoms caused by application of provocative measures. In the one case in which, on adequate trial, pyribenzamine was found to be of value in preventing or aborting urticaria induced by exposure to cold, it was necessary, after a period of 2 weeks, to increase the dose of drug used. It should be emphasized that it is not a cure for hypersensitivity to cold but that it may give symptomatic relief and deserves further study in this condition.

REFERENCES

1. Arbesman, C., Koepf, G., and Lenzner, A.: J. Allergy, **17**, 275, 1946.
2. Clark, D., Hough, H., and Wolff, H.: Experimental Studies on Headache: Observations on Histamine Headache. In Association for Research in Nervous and Mental Disease: Sensation: Its Mechanisms and Disturbances; An Investigation of the Most Recent Advances. Baltimore, Williams & Wilkins **15**, 417, 1935.
3. McElin, T., and Horton, B.: Proc. Staff Meet., Mayo Clin., **20**, 417, 1945.
4. Mayer, R.: J. Allergy, **17**, 153, 1946.
5. Mayer, R., Huttrer, C., and Scholz, C.: Science, n s., **102**, 93, 1945.
6. Pickering, G.: Clin. Sci., **1**, 77, 1933.
7. Sangster, W., Grossman, M., and Ivy, A.: Gastroenterology, **6**, 436, 1946.
8. Schumacher, G., Ray, B., and Wolff, H.: Arch. Neurol. and Psychiat., **44**, 701, 1940.

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APPLICATION OF IMMUNOLOGIC PRINCIPLES TO THE MANAGEMENT OF HAY FEVER, INCLUDING A PRELIMINARY REPORT ON THE USE OF FREUND'S ADJUVANT

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Animals exposed to infection produce in their blood and tissue fluids soluble substances which tend to prevent or cure infection. These are called antibodies since they act against the foreign agent and often confer protection or immunity against it. Antibodies do not always, however, confer increased resistance but may actually raise the susceptibility (or cause sensitization) of the host. This was shown nearly 50 years ago when Portier and Richet[39] reported that toxic extracts of the sea anemone's tentacles were tolerated by dogs in a first injection but produced violent, and often fatal, responses when reinjection was carried out some days later. To characterize this lowering of resistance, they coined the phrase "anaphylactic action" to distinguish it from its antithesis, the prophylactic or protective response. Another, and perhaps similar, type of increased susceptibility is to be seen in allergic individuals who develop, for some unknown reason related to heredity, antibodies which sensitize them to ordinarily harmless pollens. It is with this class of "pseudo-defense" reaction that the present article will deal.

Historically, the first clue to the immunologic nature of hay fever was given by the discovery of a related antigen, or foreign substance giving rise to the formation of antibodies. Although seasonal "catarrh" had been blamed upon solar light, the heat of summer, bacteria, the gouty diathesis, ozone and the emanations of flowers and plants, it was Blackley[3] who first gave scientific evidence that the last-named factor was the real cause. He not only reproduced the sneezing attacks in himself by exposure to pollen but also showed that susceptible patients reacted violently to pollen placed on the nasal, buccal or ophthalmic membranes or on the abraded skin. Thus a specific agent was shown to produce a susceptibility later called hay fever.

Despite these excellent contributions, Blackley had few followers during the next 30 years, therapy in hay fever being

directed toward the correction of "local pathology" in the nose by means of surgery, or electrocautery. In 1903 Dunbar[10] revived the pollen theory and, in an effort to duplicate the success of antitoxin in diphtheria, applied the serum of pollen-inoculated animals to the inflamed membranes of allergic subjects. Although the considerable efficacy of this therapy strengthened the concept that the disorder was related to a specific antigen, final conviction awaited the demonstration by Noon[38] in 1911 that it could be prevented by a series of preseasonal injections with pollen extract. The concept at this time was that hay fever was the result of a combination of antigen with an antibody probably of the same type as that demonstrated in sensitized animals. Since it had been shown that anaphylaxis could be prevented by means of repeated small injections during the interval between the first and the later "shocking" dose, the success of Noon's therapy was explained on a similar basis and his procedure referred to as "desensitization." It made the assumption that the hypothetical antibodies of hay fever were gradually saturated and inactivated by the administered antigen.

Ten years later, evidence for the existence of allergic antibodies was first given by Prausnitz[40] when he injected a small amount of serum from fish-sensitive Kuestner into normal skin and obtained an urticarial response to a later test with the fish extract. Although this demonstration added to the theory that hay fever and other allergic disorders were analogous to anaphylaxis in that the antigen-antibody reaction was detrimental to the host, it was not long before certain differences in the 2 states were pointed out. For example, the quality of the experimental antibodies to precipitate and to fix complement when mixed with their antigens could not be shown for the Prausnitz-Kuestner factor. The susceptibility of the latter to heating also stood in contrast to the other antibodies. By 1926 Levine and Coca[25] threw more doubt on the theory by showing, in PK (Prausnitz-Kuestner) experiments with serum from hay fever cases, that allergic sensitizing bodies were not decreased but were often increased in the circulation as the result of Noon therapy. Thus the phenomenon of desensitization, so characteristic of the experimental state, was not to be elicited in man. Schmidt and Lippard[41] later tried to reconcile the 2 schools by suggesting that the very increase in allergic antibodies might serve to bind up the antigen in the blood stream before it could reach the allergic tissues and cause distress. However, this theory is not convincing when one considers that atmospheric pollen contacts the eyes and nose *before* gaining entrance to the circulation. Although there was uncontested acceptance of the idea that hay fever was due to the union of allergic antibodies (or reagins, as Coca preferred to call them) with pollen antigen in the tissues, the mechanism underlying Noon's prevention of symptoms was unknown for years after the Prausnitz-Kuestner demonstration. Indeed, many allergists claim that it is still a mystery.

There were clues to a possible answer, however, in the European literature of the late 20's and early 30's. Van Leeuwen and Kremer[46] noted that serum from some mold-sensitive cases tended to reduce the response of skin allergic to this antigen. The following year, Jadassohn[22] described a similar neutralizing quality in ascaris-sensitive serum. It is of interest that both these observers believed that the sensitizing and neutralizing functions of their sera were properties of 1, rather than of 2, immune bodies. Shortly György, Moro and Witebsky[16] distinguished, however, between egg-allergic and egg-immune infants by means of the different zones of complement fixation shown by their sera, and Woringer[49] too referred to "deux sortes d'anticorps ovalbuminiques" in the blood of nurslings.

In 1935 Cooke, Barnard, Hebald and Stull[7] stimulated interest in the concept of 2 coëxisting types of antibody by

observing that the neutralizing quality was to be found only in sera of those of their hay fever patients who had been receiving *treatment* of the Noon variety. After a number of injections, a reduction could be found in the whealing that resulted when the patient's serum was mixed with antigen and introduced into normal skin. To explain this reduction they postulated the development, during pollen therapy of hay fever patients, of an immune factor differing from reagin (or Prausnitz-Kuestner antibody). Because this interference with whealing was noted in serum-antigen mixtures when tested in normal skin but not when injected into allergic skin, they concluded that the phenomenon was not due to combination of antigen with the new factor but to some other undetermined process. Because an antibody by definition must react demonstrably with its antigen, they referred to the hypothetical substance as a "peculiar, blocking or inhibiting type of immune antibody." Although Harley[20] concurred that the Cooke factor did not bind its antigen, it was later shown by the author[26] that the substance was an antibody and that the failure of earlier investigators to detect its antigen-binding nature was referable to their use of excessive amounts of antigen.

Within the next 2 years, the author[8] experimenting in Cooke's laboratory, succeeded in producing pollen antibodies in three normal volunteers. These antisera possessed the blocking principle described by Cooke but lacked all sensitizing power; that is, gave negative PK tests. At the same time, Cohen and Nelson[5] were independently producing the same immune body in normal sheep by means of pollen injections. Although it was natural to assume that such an antibody was formed by hay fever subjects, it was not until 2 years later that a means was found to separate it from the coëxisting reagins which had confused the results of PK tests. By means of controlled heating, the writer[26] inactivated the reagin present in the serum of treated, pollen-sensitive patients and then titrated the still active immune antibody. It was thereafter a simple matter to compare the antigen-combining quality of such heated sera with that of specimens taken from the immunized, non-allergic volunteers. No difference could be detected between the two.

The newly found antibody was formed with regularity in all subjects, whether allergic to the antigen or not. It bound and inactivated its pollen antigen, as demonstrated by negative urticarial responses to pollen-serum mixtures tested in naturally sensitive as well as in passively sensitized skin. This neutralizing capacity was definite but limited, the usual skin-test reaction resulting if too much antigen was introduced into the test mixture. The antibody differed from reagins not only in its resistance to heat and in its failure to sensitize skin but also in the speed with which it disappeared from inoculated cutaneous sites. This latter characteristic had been touched upon by Langner and Kern[24] in their earlier experiments with unheated, post-treatment serum from pollinosis cases.

Adequate confirmation has been made of the existence of these immune bodies. Sherman, Hampton and Cooke[43] found that such heat-resistant antibodies were transmitted to the offspring through the cord blood whereas the reaginic bodies of the mother were unable to pass the placental barrier. Gelfand and Frank[15] noted the induced antibodies in their treated hay fever subjects with regularity, as did also Maunsell.[37] Whereas these investigators employed modifications of the passive transfer technique employed by the writer,[26] Scully and Rackemann[42] arrived at the same conclusion by the use of the older method of Cooke wherein not only the thermostable factor but also the unheated reaginic components of the serum contribute to the end-points.

Recently another procedure for measuring thermostable antibodies in human serum has been suggested by Hampton, Johnson, Alexander and Wilson.[19] This

microprecipitin technique calls for the use of rabbit antipollen serum. The extent to which the precipitation of rabbit serum can be postponed by the presence of human thermostable antibodies serves as a measure of the latter. Presumably, the method indicates the amount of antigen which is bound and neutralized by the human serum. As in the instance of the passive sensitization type of titration, there appears to be a successful competition by the thermostable antibody for the antigen which is thereby prevented from uniting with the rabbit antibodies. In several reports, evidence for the presence of the neutralizing, heat-stable antibodies in the treated ragweed-sensitive patients has been given by this method.[1,2]

Although Cohen and Weller[6] believed that they could distinguish post-treatment serum from that of the untreated patient, Swineford[45] has not been able to substantiate their claims using the same, collodion particle method of agglutination.

The existence of these 2 different types of antibody in man has, however, been observed by workers investigating other antigens. Lowell[35,36] deduced that antibodies similar to pollen-reagins accounted for his patient's urticaria, bronchial constriction and collapse after taking insulin, whereas thermostable, neutralizing factors were responsible for her periods of insulin-fastness. Similar antibodies have been found by the author[32] in a woman who was exquisitely allergic to insulin. As graduated doses of insulin gradually built up a high level of clinical tolerance, her blood and tissues gave evidence of acquiring neutralizing antibodies which proved to be heat-resistant. When therapy was interrupted, this immunity was lost and a small dose of insulin led to extreme urticarial eruption and collapse. Her reagins remained relatively unchanged throughout the period of study. In addition to insulin, a number of other antigens have been noted by us to produce the 2 varieties of immune response. Among these are crystalline ovalbumen, bovine lactalbumen, beef and lamb, silk, and animal danders.

That this dual antibody relationship is not confined to man has been brought out by Wittich[48] in the case of dogs and by Weil and Reddin[47] for cattle. Whereas their animals showed spontaneous pollen allergy, numerous other workers have been able to induce the 2 varieties of immune body in rabbits and guinea pigs.

The clinical rôle of thermostable antibodies in man has proven a most difficult matter to determine, in spite of the strong logic in the idea that a factor capable of neutralizing a foreign irritant should be of protective value to its host. At the outset of exploring this possibility, it was necessary to learn whether the antibodies were present in the tissue fluids as well as in the circulation; for it was not to be expected that immunity distant from the allergic portals of entry would prove of much worth in preventing hay fever. Although it was the accepted belief that successfully treated patients showed no loss of skin sensitivity, delicate threshold tests revealed that this was a misconception.[27] Not only in the skin but also in the conjunctiva, it could be shown that the tolerance rose or subsided as the titer of thermostable antibody in the patient's blood was increased by Noon's therapy or was allowed to fall by discontinuing the pollen injections. This relationship between circulating and fixed antibodies was confirmed by Sherman.[44] The assumption that the induced antibodies played some rôle in the patient's protection against the atmospheric pollen was strengthened decidedly by this finding. This assumption is, however, strongly contested as the following comments will indicate.

When the antibody was still in the hypothetical stage, the Cooke group[7] were enthusiastic about their success with transfusions of blood taken from treated cases of hay fever, and spoke of a "transferable protective substance." Langner and Kern[24] noted a similar reduction in symptoms when untreated indi-

viduals were injected intramuscularly with 50 cc. of serum drawn from immunized patients. Our own transfusions of blood or serum taken from highly immune, normal subjects who lacked the reagins present in earlier experiments proved to be promptly effective in acute hay fever.[34] Attempts to correlate the titer of thermostable antibody with the relative freedom from hay fever noted among groups of patients have, however, been less encouraging. Although Maunsell[37] related seasonal resistance to the concentration of her patient's antibody, neither Scully[42] nor Gelfand[15] have detected any such connection. Among others, Cooke[9] is now of the opinion that the thermostable antibodies are not the important factor in clinical immunity.

These discouraging results should not, however, lead anyone to abandon the theory of protective antibodies in allergy. There is no other reasonable explanation. It is clinically well known that the protection resulting from injections is specific. This fact requires the assumption of a specific nature of the protective mechanism. In other words, as Coca[4] points out, "the protective mechanism must neutralize either the sensitizing antibody or the antigen." Since the sum total of pollen antigen given during a course of treatment is sufficient to neutralize only a small fraction of the reagins and since it is well established that reagins are not diminished by therapy, the relief of hay fever cannot be attributed to desensitization. This situation leaves, to continue the quotation, "only one other conceivable explanation of the phenomenon we are discussing: namely, that it is the result of the formation of neutralizing immune antibodies."

There are, in addition to such theoretical arguments for the existence of a protective antibody in hay fever, a number of practical reasons why a correlation between antibodies and clinical control might be extremely difficult to demonstrate. For example, all investigators (except Alexander) who have attacked the problem have limited themselves, to date, to the method of passive sensitization. These techniques are apt to produce inconstant results except after long experience. Furthermore, the end-points obtained vary decidedly with the test subject chosen, and this fact has been disregarded by everyone. Scully neglected to remove a variable from her studies when she titrated her sera in the unheated state. In contrast, Maunsell was dealing with thermostable components only when she determined the pollen-binding power of her patient's serum by means of tests in his own skin. We found this "auto-transfer" method very satisfactory some years ago.[26] The influence of the "test-subject" under such circumstances would not be expected to confuse the issue in the manner of a borrowed test-subject, since any host factors affecting the serologic titration would likewise probably be reflected in the clinical outcome.

Another, less important, variable in the serologic technics is the factor of ageing of the test-antigens. This has not been given adequate attention.

Far more significant than these variables are others referable to the patient himself. Neither the external environment (with reference to atmospheric pollen and many other influential factors) nor the internal environment (including the concentration of reagins, and innumerable physical and mental factors contributing to the end-result in hay fever) can be held to be alike in any 2 patients. Just as there is a range of reactions in most biologic mechanisms, such as the response to drugs, hormones like insulin, antiserum and a host of other agents, so also one would anticipate that 2 individuals with identical amounts of thermostable antibody might show somewhat different behavior during the pollinating season.

With this biologic variation in mind, we sought to reduce the complexity of the situation by concentrating on the individual rather than on groups as had been the case with other investigators seeking correlation between serologic and

clinical immunities. Among 52 patients observed for a number of years, it was found[29] that a tendency was present for symptoms to be milder when the blood titer was high than when it was lower.

Further simplification has been attained by replacing the serologic procedure with direct conjunctival testing of the patient. The undesired influence of the borrowed test-subject was thereby replaced by the desired effect of native host factors on the outcome of the titration. As anticipated, the level of conjunctival response which was associated with optimal clinical protection was found to vary with the individual, but these differences were not as great as those encountered in passive sensitization procedures. By thus reducing the variables in the test, it became evident, even in making comparisons among a group, that the higher conjunctival thresolds of 95 subjects were usually associated with the better clinical results.[33] The correlation was not sufficiently close, however, to permit of accurate predictions in advance of the season of pollination in the instance of newly acquired patients. Once the conjunctival threshold for a given individual had been determined in relation to a successful season, it could be used as a satisfactory index to future immunization. We are continuing to accumulate this type of ophthalmic and clinical data with a view to making statistical analysis of groups segregated according to underlying sensitivity. At present there is adequate population in only 1 class of sensitiveness, that of patients whose eyes respond to *small* test-doses of antigen (after they have been given a respite from injections for at least 10 months). Among 77 such equally allergic subjects, a tendency has been noted for the median hay fever hours to be progressively fewer as the conjunctival thresholds were found higher after therapy. By segregating cases in this way, it should prove possible to minimize the influence of at least 1 of the biologic variables—that of sensitizing antibodies—and thereby to increase the significance of group studies.

When more controlled means of testing the relationship between conjunctival reactivity and clinical susceptibility are evolved, it may well be found that the tolerance of the eye reflects the general immune status of the patient with considerably more reliability than can now be claimed. The basis for this statement is our experience with the eye test as a guide to the tolerance for injected antigen. For the past 5 years, it has been our practice to give each patient as much pollen antigen as he could take at every visit. Recent comparisons between the speed of therapy and the degree of ophthalmic sensitivity noted prior to the course have revealed a relationship between the 2. Although a range of speeds has been tolerated by the various members of each eye test group, the median values reveal a steady improvement in the capacity to take antigen as the conjunctival sensitivity preceding the course is lower. By referring to a curve constructed for each conjunctival class of initial sensitiveness, it is now possible to see what proportion of patients with a given degree of allergy have tolerated different paces of immunization. It is our custom to start a new patient on that booster schedule which has been safely taken by three-quarters of the patients in his eye class. This demonstrated correlation between the conjunctival and the systemic susceptibility makes it likely that a similar connection may eventually be found to exist between the eye classification at the end of treatment and the patient's behavior toward atmospheric pollen. A testing chamber with controlled pollen atmosphere would aid in the exploration of this prospect.

The attempts of Alexander[1,2] to find a relationship between thermostable antibodies and clinical relief were at first fruitful and later discouraging. Some of the difficulties enumerated above for the biologic methods of titration were also present in his studies. He did, however, exclude

the test-subject variable by using the Hampton precipitin technique.

Practical contributions to medicine have come out of the preceding theoretical investigations on the immunology of allergy. One of these is the short, or "booster," pre-seasonal treatment. The clue to this was found during the production of pollen-neutralizing antibodies in normal volunteers.[28] In order to learn whether the phenomenon of the so-called anamnestic response could be elicited, the subject was allowed a period of rest for some months and then stimulated with a large dose of antigen given in a short period. When an acceleration and heightening of antibody production resulted, it was decided to apply the principle with caution to the hay fever patient. After their initial therapy and an ensuing rest period, these subjects were able to take much faster courses than at first, and produced antibody more rapidly and more vigorously.[30]

By utilizing this "booster" principle and limiting the seasonal dose to that amount of antigen which would raise the eye response to a level known to be associated with clinical immunity, it has been found[31] that most individuals need far less therapy than is customarily given. Indeed, the average patient can be given his optimal result in as few as 7 or 8 injections and with as little as 0.1 mg. of "protein" nitrogen. The safety of such intensive therapy may be insured by adjusting the rate of increase in dosage to the tolerance of the individual, as judged by initial eye test and observation of the localized responses to each injection.

This year, a means of still further curtailing the treatment of hay fever has been tried in our laboratory. It is a procedure borrowed from experimental immunologists, who for many years have sought to improve the immune responses of animals by including various adjuvant substances in their antigenic solutions. Allergists, too, have sought for synergistic effects from such substances as gelatin, alum, tannic acid precipitation of the excitant, and admixture of their allergens with oil. Recently a very successful adjuvant procedure has been described by Freund.[11,12,13] This consists of emulsifying the aqueous antigen with mineral oil containing killed acid-fast bacilli and a lanolin-like substance, Falba or Aquaphor. Antibody responses to such emulsions are strikingly heightened and prolonged, even in some instances where the tubercle bacilli or acid-fast organisms are omitted. Whereas Freund employed such antigens as horse serum, typhoid bacilli and toxoid as antigens, Friedewald[14] had equal success in his animals with the use of influenza vaccine. Omission of the acid-fast bacilli diminished the synergistic effect. Kulka and Hirsch[23] noted that the incorporation of ragweed pollen extract into Aquaphor and paraffin containing killed tubercle bacilli led to high and persistent antibody production as well as to marked sensitivity in rabbits and guinea pigs. Henle and Henle[21] are the first to have published on the use of this adjuvant method in man. Vaccination of 80 women with influenza A and B antigens, in an emulsion of mineral oil and Falba, produced higher and more sustained titers than were found among the control group given aqueous vaccine. Halbert, Mudd and Smolens[17] had notable success with Shigella antigen in mineral oil and Falba, and concluded that the presence of acid-fast bacilli was not an important factor in the effectiveness of the menstruum. In additional laboratory experiments with this simplified form of the adjuvant, these investigators[18] were able to reduce decidedly the acute toxicity of such substances as ricin, botulinus and tetanus toxins. They have not yet published their results with the use of simplified adjuvant in 70 human subjects.

These observations have stimulated the author to test the efficacy of the simplified adjuvant mixture in pollen-allergic patients. Accordingly, 14 patients were given their booster stimuli in the form of 1 injection containing from 200 to 2000 units of ragweed antigen emulsified in Falba and mineral oil; 18 others took 600 to 7250 units in 2 such subcutaneous

treatments, a few days or weeks apart. One individual was administered his usual dose of 11,000 units in 4 visits. The volume of each injestion was between 0.1 and 0.3 ml. of the emulsion.

In keeping with the reduction in toxicity noted by Halbert, there were remarkably few untoward reactions to these doses, which ranged in size from 2- to 20-fold the amount previously tolerated by the patient in aqueous form. Whereas, only 4 mild systemic responses occurred in connection with the first 33 treatments, more difficulty was encountered after the 19 second inoculations which elicited 2 slight, 1 moderate and 1 severe overdose reaction. These doses were larger than the initial ones given the same individuals by $2\frac{1}{2}$- to 6-fold. The limits of safety of the procedure were thus exposed. In all, 9 untoward responses were encountered among 54 adjuvant doses given our 33 subjects. All but 2 were of mild nature.

The immunologic responses to adjuvant antigen were gauged by the usual threshold tests of the conjunctiva, comparisons being drawn with similar data for past seasons when total preseasonal doses of 10,000 units or more had been administered in aqueous form. Whereas these past courses had improved the ophthalmic tolerances by ratios ranging from 1 to 32 with a median value of 4, the adjuvant courses have increased the reaction level of the eye by about twice these amounts, the range being 2 to 64 and the median ratio being 8.

The clinical efficacy of this new form of immunization can be gauged now that the current season of ragweed pollination is over. Among the 31 members of the adjuvant group whose clinical data are up to date, 13 give a history of having gained almost complete relief from their original symptoms. This figure is to be compared with 10, the number of excellent results recorded by them in an earlier year. A loss of at least three-fourths of their hay fever has been noted by another 8 of the subjects, which is identical with the number claiming this degree of success for a prior season. Fair responses (loss of 50 to 70% of original symptoms) were reported by 5 of the series whereas 9 of them had given a similar estimate for a preceding course. Poor outcomes, with less than half of the hay fever abolished, were described by 5 of the 31 individuals. Three of these had failed to procure relief after usual therapy. Moreover, our pollen count was 1807 in 1947, compared to 1332 in 1946 and an 18-year mean of 1109.

On the whole, it would appear that the clinical efficacy of the abbreviated therapy in adjuvant form compares favorably with that of more protracted courses calling for aqueous antigen. There has been no localized discomfort or abscess formation, as encountered in 2 of Henle's 80 patients. The incidence of untoward reaction can doubtless be reduced during future trials.

It should be emphasized in closing that this form of adjuvant therapy is in its developmental stages and is under no circumstances ready for application to office practice. Only by virtue of our intimate acquaintance with each patient's tolerance was it justifiable to administer the amounts of antigen specified above. The investigation is mentioned because of its interesting implications for the future management of allergic diseases and particularly because of its bearing on the theory of protection. This further demonstration that an immunologic procedure can bring about a clinical insusceptibility which is associated with the production of specific, neutralizing antibodies makes the concept of a protective rôle for these bodies still more valid.

REFERENCES

(1.) **Alexander, H., Johnson, M.,** and **Alexander, J.**: J. Allergy, **17**, 340, 1946. (2.) **Alexander, H.,** and **Johnson, M.**: Paper read before the Third Annual Meeting of the American Academy of Allergists, November, 1946.

(3.) **Blackley, C.**: Experimental Researches on the Causes and Nature of Catarrhus Æstivus, London, 1873.

(4.) **Coca, A.**: Ann. Allergy, **1**, 120, 1943. (5.) **Cohen, M.**, and **Nelson, T.**: J. Immunol., **34**, 63, 1938. (6.) **Cohen, M.**, and **Weller, R.**: J. Allergy, **12**, 242, 1941. (7.) **Cooke, R., Barnard, J., Hebald, S.**, and **Stull, A.**: J. Exp. Med., **62**, 733, 1935. (8.) **Cooke, R., Loveless, M.**, and **Stull, A.**: J. Exp. Med., **66**, 689, 1937. (9.) **Cooke, R.**: J. Allergy, **15**, 212, 1944.

(10.) **Dunbar, W.**: München. med. Wchnschr., **50**(1), 924, 1903.

(11.) **Freund, J.**, and **McDermott, K.**: Proc. Soc. Exp. Biol. and Med., **49**, 548, 1942. (12.) **Freund, J.**, and **Walter, A.**: Proc. Soc. Exp. Biol. and Med., **56**, 47, 1944. (13.) **Freund, J.**, and **Bonanto, M.**: J. Immunol., **48**, 325, 1944. (14.) **Friedewald, W.**: J. Exp. Med., **80**, 477, 1944.

(15.) **Gelfand, H.**, and **Frank, D.**: J. Allergy, **15**, 332, 1944. (16.) **György, P., Moro, E.**, and **Witebsky, E.**: Klin. Wchnschr., **9**, 1012, 1930.

(17.) **Halbert, S., Mudd, S.**, and **Smolens, J.**: J. Immunol., **53**, 291, 1945. (18.) **Halbert, S., Smolens, J.**, and **Mudd, S.**: J. Immunol., **51**, 39, 1945. (19.) **Hampton, S., Johnson, M., Alexander, H.**, and **Wilson, K.**: J. Allergy, **14**, 227, 1943. (20.) **Harley, D.**: J. Path. and Bact., **44**, 589, 1937. (21.) **Henle, W.**, and **Henle, G.**: Proc. Soc. Exp. Biol. and Med., **59**, 179, 1945.

(22.) **Jadassohn, W.**: Arch. f. Dermat. u. Syph., **156**, 690, 1928.

(23.) **Kulka, A.**, and **Hirsch, D.**: J. Immunol., **50**, 127, 1945.

(24.) **Langner, P.**, and **Kern, R.**: J. Allergy, **10**, 1, 1938. (25.) **Levine, P.**, and **Coca, A.**: J. Immunol., **11**, 411, 1926. (26.) **Loveless, M.**: J. Immunol., **38**, 25, 1940. (27.) **Loveless, M.**: South. Med. J., **33**, 869, 1940. (28.) **Loveless, M.**: J. Immunol., **44**, 1, 1942. (29.) **Loveless, M.**: J. Immunol., **47**, 165, 1943. (30.) **Loveless, M.**: J. Immunol., **47**, 283, 1943. (31.) **Loveless, M.**: J. Allergy, **15**, 311, 1944. (32.) **Loveless, M.**: Federation Proc., **5**, 250, 1945. (33.) **Loveless, M.**: Ann. Allergy, **3**, 333, 1945. (34.) **Loveless, M.**: Unpublished experiments. (35.) **Lowell, F.**: J. Clin. Invest., **23**, 225, 1944. (36.) **Lowell, F.**: J. Clin. Invest., **23**, 233, 1944.

(37.) **Maunsell, K.**: Lancet, **2**, 199, 1946.

(38.) **Noon, L.**: Lancet, **1**, 1572, 1911.

(39.) **Portier, P.**, and **Richet, O.**: Compt. rend. Soc. de biol., **54**, 170, 1902. (40.) **Prausnitz, C.**, and **Kuestner, H.**: Centralbl. f. Bakteriol., 1 Abt., Orig., **86**, 160, 1921.

(41.) **Schmidt, J.**, and **Lippard, V.**: J. Dis. Child., **56**, 550, 1938. (42.) **Scully, M.**, and **Rackemann, F.**: J. Allergy, **12**, 549, 1941. (43.) **Sherman, W., Hampton, S.**, and **Cooke, R.**: J. Exp. Med., **72**, 611, 1940. (44.) **Sherman, W.**: J. Immunol., **40**, 238, 1941. (45.) **Swineford, O.**, and **Houlihan, R.**: J. Allergy **18**, 190, 1947.

(46.) **van Leeuwen, W.**, and **Kremer, W.**: Ztschr. f. Immunitätsforsch. u. exp Therap., **50**, 462, 1927.

(47.) **Weil, A.**, and **Reddin, L.**: J. Immunol., **47**, 345, 1943. (48.) **Wittich, F.**: J. Allergy, **12**, 247 1941. (49.) **Woringer, P.**: Compt. rend. Soc. de biol., **109**, 206, 1932.

RADIOLOGY

UNDER THE CHARGE OF

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RHEUMATOID SPONDYLITIS

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THE work of recent investigators[1,2,5,6,9,13–15] has greatly increased knowledge of rheumatoid spondylitis and has resulted in the establishment of criteria for the early diagnosis. The purpose of this review is to emphasize these criteria. For those who are interested in the clinical aspect of the subject the works of Polley,[14] Boland and Present,[1] Boland and Shebesta,[2] Polley and Slocumb,[15] Hench, Slocumb and Polley,[9] and Dunham and Kautz[5] are especially recommended.

The diagnosis of early rheumatoid spondylitis reflects progress in radiology since roentgenologic observations often contribute important information which may lead to the diagnosis being made before the symptoms are conspicuous and it is not infrequent that the roentgenologic diagnosis of early rheumatoid spondylitis is made before that condition has been suspected clinically. Clinicians who are especially interested in this disease can recognize it by the symptoms alone very quickly, but physicians who are not so well acquainted with it not infrequently fail to recognize the indefinite symptoms of early rheumatoid spondylitis. Therefore the roentgenologist can be of great service by recognizing the early roentgenologic manifestations of the disease and suggesting the presence of that condition to the clinician.

Confusion in terminology has led to misunderstanding with regard to rheumatoid spondylitis. There are not many types of arthritis of the spinal column. Hypertrophic arthritis or osteo-arthritis of the spinal column is not the result of infection or inflammation but merely of wear and tear. Infectious arthritis due to certain specific infections is usually localized to a few segments of the spinal column; the infections include tuberculosis, brucellosis, typhoid and pyogenic infections. Tabetic arthropathy falls in a special category. Rheumatoid spondylitis is the only other true arthritic process affecting the spinal column and it is the only one, aside from osteo-arthritis, which may affect the entire spinal column. The use of numerous synonyms has led to considerable confusion. Polley has assembled the following synonyms for rheumatoid spondylitis: (1) spondylitis ankylopoietica (Fraenkel); (2) spondylitis ossificans ligamentosa (Knaggs); (3) spondylitis rhizomelique (Marie); (4) spondylarthritis ankylopoi; etica; (5) spondylitis ankyloarthritica-(6) spondylitis adolescens (Scott); (7) vertebral type of arthritis deformans (Osler); (8) spondylitis deformans (common term in England); (9) ankylosing polyarthritis (Oppel); (10) fibrositis ankylopoietica dorsi (Krebs); (11) syndesmitis ossificans; (12) spondylitis atrophica ligamentosa; (13) Marie-Strümpell's disease; and (14) von Bechterew's disease.

Naturally the use of so many different terms has not aided in clarifying the subject. The early investigators did not have the advantage of roentgenologic observations and, therefore, their understanding of the disease was necessarily the more incomplete. This applies especially to the former belief that there were 2 types of rheumatoid spondylitis: the Marie-Strümpell type that was supposed always to begin in the lumbar region, and the von Bechterew type which was supposed to start in the thoracic region. There is

no reason to divide rheumatoid spondylitis into 2 types. According to most observers the disease almost always starts in the sacro-iliac joints and the lumbar apophyseal joints and then progresses upward. Only rarely is the thoracic portion of the spinal column involved when the sacro-iliac joints and lumbar portion are not involved also. In an endeavor to clarify the situation the American Rheumatism Association has adopted the term "rheumatoid spondylitis;" the use of that term by all investigators is most earnestly recommended.

Hench, Slocumb and Polley[9] have defined rheumatoid spondylitis as an inflammatory disease of unknown origin which generally begins in the sacro-iliac joints and progressively involves the apophyseal joints of the spinal column, the paravertebral muscles and ligaments and the costovertebral and costochondral junctions. Inflammation, destruction and, as a rule, ankylosis of the sacro-iliac joints occur. Synovitis with destructive arthritis of the apophyseal joints is followed by ankylosis. Painful spasms and contractures of the paravertebral muscles occur. Calcification of the paravertebral ligaments is seen. Sometimes the hips and, less often, other joints of the extremities are involved. The shoulders are affected only occasionally. Hence rheumatoid spondylitis is chiefly a disease of the spinal column or torso.

As the term "rheumatoid spondylitis" implies, this condition is thought by many to be related to rheumatoid arthritis. This relationship has not been definitely established and it is still the subject of considerable controversy. The arguments favoring and opposing this concept have been summarized by Hench, Slocumb and Polley.[9] In opposition to the concept are the following points: (1) The sex incidence of the 2 diseases is different. Rheumatoid arthritis is 2 or 3 times more frequent in women than in men. Rheumatoid spondylitis is 9 times more frequent in men than in women. (2) The age incidence also is different. Rheumatoid arthritis develops most frequently in persons who are between the ages of 25 and 40 years. Rheumatoid spondylitis develops usually in persons who are between 15 and 30 years of age. (3) Calcification of ligaments is rare in rheumatoid arthritis and common in rheumatoid spondylitis. (4) Subchondral rarefaction occurs in rheumatoid arthritis whereas subchondral osteitis is seen in rheumatoid spondylitis. (5) Subcutaneous fibrous nodules are found in rheumatoid arthritis but not in rheumatoid spondylitis. (6) Iritis is 2 or 3 times more common in rheumatoid spondylitis than in rheumatoid arthritis. (7) The concentration of agglutinins of hemolytic streptococci in the blood is lower and that of plasma phosphatase is increased more often in rheumatoid spondylitis than in rheumatoid arthritis. (8) More or less complete remissions are more common in rheumatoid spondylitis than in rheumatoid arthritis. (9) Chrysotherapy is effective in a considerable percentage of the cases of rheumatoid arthritis but is of no value in rheumatoid spondylitis. (10) Roentgen therapy is of value in rheumatoid spondylitis but is not useful in the treatment of rheumatoid arthritis.

Favoring the concept that rheumatoid spondylitis is the spinal equivalent of rheumatoid arthritis are the following points: (1) In cases of rheumatoid spondylitis arthritis of peripheral joints sometimes develops which is clinically indistinguishable from peripheral rheumatoid arthritis without spondylitis. (2) The pathologic reactions in the affected hips and peripheral joints of rheumatoid spondylitis are identical with those of rheumatoid arthritis without spondylitis. (3) In certain cases in which peripheral joints are affected first, classic rheumatoid spondylitis later develops. (4) The pathologic reactions in the vertebral apophyseal joints of patients who have rheumatoid spondylitis are said to resemble those of classic rheumatoid arthritis without spondylitis.

The pathologic processes that are present in rheumatoid spondylitis have been

studied histologically insofar as affected apophyseal joints of the spinal column and involved peripheral joints are concerned. As yet pathologic studies of the sacro-iliac joints in the active stage of the disease have not been reported; the only ones reported have been made in the late stage after ankylosis has occurred. Specimens taken from affected apophyseal spinal and peripheral joints in cases of rheumatoid spondylitis have shown the changes identical with those seen in rheumatoid arthritis without spondylitis.[2,14] According to Dunham and Kautz,[5] Guntz found that in the apophyseal joints of the spinal column in the early stages of the disease there was inflammation of the joint surfaces with exudation, invasion of the cartilage with round cells and production of connective tissue. After further progress of the disease only islands of cartilage remained and finally ankylosis occurred. Early in the disease all of the apophyseal joints of the spinal column were not involved but the process was never limited to 1 or 2 joints. He found the same pathologic process in the costovertebral joints. According to Boland and Shebesta,[2] J. E. Flynn stated that pathologic specimens taken from apophyseal joints during the active phase of rheumatoid spondylitis revealed microscopic findings similar to those seen in peripheral rheumatoid arthritis.

Rheumatoid spondylitis may occur in persons of any age according to Polley and Slocumb.[15] Their youngest patient was 4 years of age and the oldest 63 years of age. They found the average age of onset to be 26.7 years. In 80% of their cases the symptoms first appeared when the patients were between 15 and 35 years of age. Men were affected 9 times more frequently than women. Thus it is obvious that rheumatoid spondylitis is a disease that affects young men predominantly. The importance of this is shown by the observation of Boland and Present[1] that 18% of the patients with chronic back complaints who were admitted to an army general hospital were found to have rheumatoid spondylitis. They also noted that, although in civilian life the ratio of rheumatoid spondylitis to rheumatoid arthritis is 1/6, according to the best figures, in the Army the relative incidence was 1/3 or 1/2. This they attributed to the fact that rheumatoid spondylitis has a predilection for men of military age and that strenuous physical exertion brought to light cases of mild or early rheumatoid spondylitis, including many cases in which the disease was present before the patient was inducted into the Army but was not recognized.

Far-advanced rheumatoid spondylitis can be recognized with ease but when the disease is this extensive it does little good to make the diagnosis, since little can be done in the way of treatment. The diagnosis must be made early in the course of the disease if benefit is to be expected from treatment. Many of the early symptoms are vague and indefinite, but a few may be objective. The unwary physician may easily overlook the true nature of the condition. Boland and Present[1] noted that in many cases the condition was mislabeled as lumbago, fibrositis, muscular rheumatism, chronic low back strain, renal disease or idiopathic sciatica. Krebs[11] found that because definite objective findings are absent early in the disease, patients were often considered malingerers or neurotic. Polley[14] found that in 41 of the 1035 cases he studied operation had been performed at one time or another because the symptoms were not recognized as those of early rheumatoid spondylitis but were attributed to some condition requiring an operation. The surgical procedures that he listed are most interesting and reveal the deceiving nature of rheumatoid spondylitis. They were: operations for protruded intervertebral disks; spinal fusions; laminectomy; biopsy of hip for suspected tuberculosis; synovectomy of knee and ankle; surgical drainage of the knees; arthrodesis of foot and ankle; removal of piece of bone from foot; abdominal fasciectomy to correct posture; fasciotomy of

hips; removal of calcaneal bursæ bilaterally; removal of bilateral calcaneal spurs; appendectomy; appendectomy and cholecystectomy; hysterectomy and appendectomy; cholecystectomy; thyroidectomy; cholecystectomy and tonsillectomy; repair of right inguinal hernia; appendectomy, salpingectomy and oöphorectomy; hysterectomy and oöphorectomy. Almost none of the patients received any benefit as result of the operation. Polley[14] also found that in 10% of his cases some anomaly of the spinal column was present such as sacralization of the last lumbar vertebra, spina bifida occulta, spondylolisthesis, cervical rib or anomalies of the sacrum and coccyx. In some cases these anomalies probably were considered the cause of the symptoms that were present.

It is obvious, therefore, that the early symptoms of rheumatoid spondylitis are often misleading and that a review of these symptoms and the methods of early diagnosis are warranted. Especially is it pertinent that this disease be discussed more frequently at present, as the operation for the removal of the protruded intervertebral disk has become a frequent remedy for the relief of low backache and sciatica and rheumatoid spondylitis may mimic the syndrome of protruded intervertebral disk.

THE DIAGNOSIS OF EARLY RHEUMATOID SPONDYLITIS. The diagnosis of early rheumatoid spondylitis is not too difficult, as a rule, if that condition is thought of and considered as a cause of the patient's symptoms. But all too frequently rheumatoid spondylitis is not even considered. For this reason the early symptoms of this condition must become more widely known by physicians. Boland and Shebesta[2] have ably described these symptoms:

"Because the disease almost invariably begins in the sacro-iliac joints, the symptoms and findings referable to the lower back at onset may be considered as expressions of sacro-iliac involvement. In approximately 75% of cases the early symptoms consist of aching and stiffness of the lower back, which qualitatively exhibit the characteristics of 'fibrositis,' that is, are most pronounced on arising in the morning, are accentuated by physical inactivity and ameliorated by mild exercise, are subject to fluctuations with weather changes, and are relieved temporarily by local heat and salicylates. At first, these symptoms may be intermittent, but after several months or a few years they tend to become persistent. Transient sharp pains or 'catches' in the lower back or less well-defined complaints, as constant dull discomfort, a soreness, or a tired feeling (accompanied or unaccompanied by symptoms of 'fibrositis') may also indicate sacro-iliac involvement. In 10 to 15% of cases sciatica, often intermittent and alternating from side to side, accompanies the phase of sacroiliitis.

"The back may be entirely normal on examination. In about 50% of patients with active sacroiliitis, tenderness on percussion may be elicited over 1 or both sacro-iliac joints. Orthopedic tests causing motion in the sacro-iliac joints may induce pain. Mild muscle spasm in the lumbar region without true restriction of motion is common.

". . . When the disease is active in the lumbar, thoracic and cervical regions, the dominant symptoms consist of pain, aching, stiffness, and restriction of motion in the involved regions. Sciatica is common with lumbar involvement, girdle pains are frequent when the thoracic spine is affected, but cervical radicular pain is rare. Lumbar, thoracic, or cervical involvement may be identified by the presence of the following general signs in the respective regions of the back: limitation of motion; persistent paravertebral muscle spasm; persistent tenderness to percussion over and just lateral to the spinous processes; pain on forced motion of the spine; paravertebral muscle atrophy. Involvement of the lumbar spine is further characterized by straightening of the normal lordotic curve and by muscle atrophy in the lower portion of the lumbar segment, giving this area an 'ironed-out' appearance. Thoracic involvement may be further identified by chest pain on deep inspiration, restricted respiratory excursion, flattened anterior chest, and thoracic kyphosis. A characteristic protruded position of the neck may develop when the cervical spine is involved.'

Polley[14] emphasized the tendency for exacerbations and remissions as well as

the shifting character of the early symptoms of rheumatoid spondylitis. He further noted that sciatica was the first symptom in 10% of cases and that in 10% more it developed later in the course of the disease. This is of great importance since physicians often make the diagnosis of protruded intervertebral disk when sciatic pain is present. Polley also found that in 23.4% of his cases the first symptoms indicated disease of the peripheral joints and these tended to disappear without any apparent residual damage to the joint.

The sedimentation rate is a helpful laboratory test. It is in no way specific, but if the rate is abnormally high it does indicate that the patient's symptoms are not psychogenic and that an active pathologic process is present. Boland and Shebesta[2] have made it clear, however, that although the sedimentation rate fairly consistently gauges the activity of rheumatoid spondylitis and roughly parallels the severity of the disease, in 15 to 20% of cases of mild but clinically active rheumatoid spondylitis it is normal. Polley and Slocumb[15] also found the sedimentation rate normal in nearly a fifth of their cases. Therefore it must be kept in mind that although an abnormally high sedimentation rate may be evidence in favor of rheumatoid spondylitis, a normal sedimentation rate does not exclude that disease. Patients who have rheumatoid spondylitis often have moderately severe, hypochromic anemia, but this is not of much importance in diagnosis.

Roentgenologic Diagnosis. Roentgenologic manifestations are of prime importance in the diagnosis of early rheumatoid spondylitis. The roentgenologist's help in the diagnosis of this disease may be in 1 of 2 ways: He may suggest the presence of rheumatoid spondylitis in cases in which that condition is not suspected clinically. This occurs not infrequently and is of inestimable value to the clinician. Secondarily, he can confirm the clinical impression that rheumatoid spondylitis is present. Without confirmation by roentgenologists, many clinicians are reluctant to make a definite diagnosis of rheumatoid spondylitis but prefer to watch the patient closely until roentgenologic evidence of the condition is present. As mentioned previously, however, those who have had great clinical experience with this disease at times can make an accurate diagnosis before changes are apparent in the roentgenograms.

The roentgenologic diagnosis of early rheumatoid spondylitis depends primarily on changes that are seen in the sacro-iliac joints. The importance of the destructive changes seen in the sacro-iliac joints was first emphasized by Krebs,[11] Krebs and Vontz,[12] and Scott,[16] and later verified by Forestier.[6] Other investigators[1,2,5,9,14,15] have concurred recently in the belief that the changes in the sacro-iliac joints are the most important roentgenologic manifestations of the disease. Hench, Slocumb and Polley[9] stated: "The most useful single laboratory aid in the diagnosis of rheumatoid spondylitis consists in roentgenographic examination of sacro-iliac joints. Although there is an appreciable lag between the development of physical findings and roentgenographic alterations, this lag is generally more than counterbalanced by the patient's tardiness in consulting a physician. Hence, even in fairly early cases roentgenograms generally already reveal sacro-iliac alterations."

In 1934, Scott[16] found that in 110 cases of rheumatoid spondylitis roentgenologic evidence of sacro-iliac involvement was present in all. Polley[14] found that in 98% of 1035 cases of rheumatoid spondylitis the sacro-iliac joints as shown roentgenologically were involved. Similarly Boland and Present[1] found that sacro-iliac changes had occurred in all of the 100 cases that they studied. They even hesitated to make a clinical diagnosis of rheumatoid spondylitis if the sacro-iliac joints appeared normal. Forestier[6] found that there was no demonstrable sacro-iliac involvement in only 2 of 135 cases, whereas in 12 cases changes in the sacro-iliac joints were not associated with roentgenologic

evidence of vertebral involvement. Cases were reported by Buckley[4] and Gordon[7] in which there was no roentgenologic evidence of sacro-iliac disease. Key and associates[10] studied the bones of 2 patients with rheumatoid spondylitis who died and who had vertebral involvement but who had no changes in the sacro-iliac joints. Oppenheimer[13] and Borak,[3] although recognizing that changes in the sacro-iliac joints were frequent, stated that the diagnosis must be based on roentgenologic evidence of alteration in the vertebral apophyseal joints.

Most investigators have found that the roentgenologic demonstration of disease of the apophyseal joints of the spinal column is difficult and not reliable as a basis for the diagnosis of early rheumatoid spondylitis. The apophyseal joints must be studied by means of roentgenographic views taken at special angles and since the plane of the apophyseal joints varies greatly, it is often necessary to take several views at different angles. Oppenheimer,[13] who has contributed much to our knowledge of changes in the apophyseal joints, mentioned the difficulty of demonstrating these changes roentgenologically. Boland and Shebesta[2] found changes in the apophyseal joints in only 9 of their 50 cases, and Boland and Present[1] never found the apophyseal joints involved unless the sacro-iliac joints were affected also. If the roentgenologist is to find evidence of rheumatoid spondylitis in patients in whom this disease is not suspected clinically, he must do this by means of routine roentgenographic views and, therefore, he most certainly must depend on the changes in the sacro-iliac joints rather than those in the apophyseal joints.

The earliest changes of rheumatoid spondylitis that occur in the sacro-iliac joints are not always easy to recognize in the roentgenogram. These do not consist of marginal sclerosis, as some investigators have stated, but instead, as Forestier noted, the contour of the subchondral bone of the sacro-iliac joint becomes indistinct and hazy; the joint loses its clear-cut margins and becomes indistinct. This marginal decalcification causes the joint to appear widened. Somewhat later sclerosis begins to appear at the margins of the joints. This osteosclerosis may be spotty and mottled and at this stage the margins of the joint are even more indistinct. The process gradually progresses until there is loss of the joint space and fibers of bone are seen growing across this space. These osseous fibers are the first evidence that ankylosis is developing and this process progresses until ultimately there is smooth bony ankylosis of the joints. If the diagnosis of rheumatoid spondylitis is to be made early in the course of the disease, the roentgenologist must search carefully for those early manifestations of the disease which consist of decalcification at the margins of the joint with resultant indistinct outlines of the joint. A moderate degree of marginal sclerosis occurs fairly early many times but dense sclerosis of the bone and ankylosis are late manifestations of the disease. At times only 1 sacro-iliac joint will show evidence of disease; Polley[14] noted this unilaterality in 6 of his 1035 cases.

Roentgenologic evidence of vertebral involvement almost always appears much later than that of sacro-iliac involvement. In routine lateral roentgenologic views the first apparent change in the vertebræ indicative of rheumatoid spondylitis is a peculiar "squaring-off" of the vertebral bodies. This change usually is evident in the thoracolumbar region first and it is due to fine calcification of the anterior longitudinal ligament which causes a filling in of normally concave anterior vertebral surfaces and "squares off" the anterior vertebral angles. Later in the course of the disease calcification of the vertebral ligaments is more obvious and appears in the roentgenogram as fine lines of calcification which cross from the margin of one vertebral body to the next. At first this process can be seen only in 1 or 2 interspaces, usually in the lumbar region. Later the ligamentous calcification becomes dense and more extensive

and gradually involves all the vertebral ligaments until the entire spinal column is involved. The end-result is the so-called bamboo spine.

Although involvement of the apophyseal joints of the spinal column occurs earlier than vertebral calcification, changes in them are more difficult to see when routine views are used and as stated before, even when views taken at special angles are used the alteration in these joints still cannot be seen many times. The earliest change in these joints is decalcification of the articular surfaces which then appear indistinct in the roentgenogram. Marginal osteosclerosis can be seen at times and this process may progress to ankylosis, as in the sacro-iliac joints. Osteoporosis of the vertebræ is almost always seen after the spinal column has begun to be immobilized, but this is not an early manifestation of the disease. The intervertebral disks are not involved early but at times very late in the course of the disease there may be involvement of an occasional interspace; the vertebral surfaces become irregular and indistinct. Also as a late manifestation the costovertebral articulations may be involved and ankylosis is the end-result. The symphysis pubis may be affected and not infrequently the ischial tuberosity will show irregularity and bony proliferation. This last change is probably the result of involvement of the adjacent ischial bursæ by the inflammatory process.

Occasionally definite roentgenologic evidence indicates that the disease has affected 1 or both hips. The appearance is identical with that caused by rheumatoid arthritis with narrowing of the joint space and decalcification and indistinctness of the articular surface. In a few cases other peripheral joints will show similar roentgenologic evidence of involvement.

Differential Diagnosis. Changes in the spinal column due to hypertrophic arthritis, so-called osteo-arthritis, can be distinguished from those of rheumatoid spondylitis with ease as a rule. As Forestier[6] has noted, osteophytes have a thick base, are covered with a cortex which comes from that of the vertebral body and have a structure of cancellous bone like the vertebral body itself. The syndesmophytes of rheumatoid spondylitis are clear-cut, linear calcifications without a cortex and look like a thin or thick comma added to the contour of the vertebral body. Dunham and Kautz[5] listed the following characteristics of osteo-arthritis: (1) it occurs after the 4th decade of life in 80% of cases; (2) the sedimentation rate is not elevated; (3) fever and leukocytosis are uncommon; (4) degeneration of the intervertebral disks is present; (5) osteophytes, but no syndesmophytes are present; (6) wedging of the vertebral bodies occurs; (7) there may be true bony bridging between vertebral bodies; (8) occasionally there is coarse calcification on the anterior surface of the vertebral column in the thoracic region. Destructive changes never are seen in the sacro-iliac joints in this condition. Ankylosis of the sacro-iliac joints are found not infrequently as a senile change but not as the result of infection. Hypertrophic changes are seldom seen in the sacro-iliac joints except as the result of abnormal weight bearing as in marked scoliosis and then only 1 sacro-iliac joint is affected as a rule and the rather sharp marginal osteitis does not resemble that produced by rheumatoid spondylitis.

A condition, known as "osteitis condensans ilii," was described, according to Hare and Haggart,[8] by Sicard, Gally and Haguenau in 1926. Hare and Haggart[8] recently reported cases and reviewed the subject. It has only 1 resemblance to rheumatoid spondylitis and that is that osteosclerotic changes appear in the sacro-iliac joints. This condition is found almost exclusively in women. Roentgenologically dense sclerotic bone may obliterate the trabeculæ in the articular portion of the ilium and the portion of the ilium adjacent to the sacro-iliac articulations. The demarcation between normal and abnormal bone is distinct. The involve-

ment is usually bilateral but it has been reported in only 1 joint. The etiology is unknown. The condition is rare, but when it does occur, it is frequently during or after pregnancy. It is characterized by recurrent attacks of chronic low backache. The sedimentation rate is never elevated. Its resemblance to rheumatoid spondylitis is superficial.

Unilateral destructive changes in the sacro-iliac joints due to tuberculosis or osteomyelitis are seen at times. There is not much resemblance to rheumatoid spondylitis when this occurs.

The roentgenologic manifestations of rheumatoid spondylitis, therefore, appear distinctive and do not seem to resemble closely those of any other conditions which involves the spinal column and sacro-iliac joints. The most important factors in the roentgenologic diagnosis of early rheumatoid spondylitis are close scrutiny of the sacro-iliac joints and the ability to recognize the early manifestations of the disease as it affects these joints.

Boland and Present[1] have listed the following conditions under which rheumatoid spondylitis should be suspected and it would be well if all physicians were well acquainted with these.

"1. Suspect rheumatoid spondylitis when a young man complains of chronic recurrent or persistent low back aching and stiffness, with or without catching pains, especially if the sedimentation rate is elevated.

"2. Suspect rheumatoid spondylitis in the young man who complains of such vague symptoms as a 'tired feeling' in the lower part of the back on standing and walking, persistent low back soreness, silent restriction of back motion, or indefinite sharp pains in the buttocks, hips or lower part of the back, especially if accompanied by an elevated sedimentation rate or general constitutional symptoms.

"3. Suspect rheumatoid spondylitis in all cases of sciatica in young men, particularly if recurrent or alternating from side to side or associated with aching and stiffness of the lower back.

"4. Suspect rheumatoid spondylitis in patients with thoracic girdle pains, especially if accompanied by symptoms in the lower part of the back.

"5. Suspect rheumatoid spondylitis when persistent back symptoms develop in a patient with peripheral rheumatoid arthritis.

"6. In the absence of roentgenographic evidence of sacro-iliac involvement an unequivocal diagnosis of rheumatoid spondylitis should not be made unless characteristic changes are present in the apophyseal joints.

"7. Remember that characteristic Roentgen ray changes in the sacro-iliac or apophyseal joints may not develop for months after the onset of symptoms. Do not eliminate the possibility of rheumatoid spondylitis on negative Roentgen rays alone unless persistent symptoms have existed for at least 3 years.

"8. Definite bilateral destructive and/or sclerotic changes in the sacro-iliac joints, noted roentgenographically, almost invariably indicate rheumatoid spondylitis.

"9. Be cautious in making a diagnosis of rheumatoid spondylitis with unilateral sacro-iliac involvement unless other characteristics of the disease are present or unless peripheral rheumatoid arthritis coëxists. Persistent unilateral sacroiliitis may be due to tuberculosis.

"10. Calcification of the paravertebral ligaments may result from several causes and in itself is not sufficient evidence for the diagnosis of rheumatoid spondylitis; changes in the apophyseal and/or sacro-iliac joints must also be present.

"11. Remember that the sedimentation rate may be normal in 15 to 20% of cases with active disease and that constitutional symptoms are usually milder than in rheumatoid arthritis involving peripheral joints.

Summary. The symptoms of early rheumatoid spondylitis are often misleading. As a result the symptoms often are either dismissed as unimportant or are attributed to some other disease.

Roentgenologic manifestations of early rheumatoid spondylitis are often sufficiently definite so that their presence alone provides sufficient evidence for a definite diagnosis of that condition or for confirmation of the clinical impression that rheumatoid spondylitis is present.

Destructive changes in the sacro-iliac joints are definite evidence of rheumatoid spondylitis, especially when there is bilateral involvement.

REFERENCES

(1.) **Boland, E. W., and Present, A. J.**: J. Am. Med. Assn., **129**, 843, 1945. (2.) **Boland, E. W., and Shebesta, E. M.**: Radiology, **47**, 551, 1946. (3.) **Borak, J.**: Radiology, **47**, 128, 1946. (4.) **Buckley, C. W.**: Quoted by Hench, P. S., Slocumb, C. H., and Polley, H. F.[9]
(5.) **Dunham, C. L., and Kautz, F. G.**: Am. J. Med. Sci., **201**, 232, 1941.
(6.) **Forestier, J.**: Radiology, **33**, 389, 1939.
(7.) **Gordon, R. G.**: Quoted by Hench, P. S., Slocumb, C. H., and Polley, H. F.[9]
(8.) **Hare, H. F., and Haggart, G. E.**: J. Am. Med. Assn., **128**, 723, 1945. (9.) **Hench, P. S., Slocumb, C. H., and Polley, H. F.**: Med. Clin. North America, **31**, 879, 1947.
(10.) **Key, J. A., Rosenfeld, H., and Tjoflat, O. E.**: Quoted by Hench, P. S., Slocumb, C. H., and Polley, H. F.[9] (11.) **Krebs, W.**: Fortschr. a. d. Geb. d. Röntgenstrah., **50**, 537, 1934. (12.) **Krebs, W., and Vontz, O.**: Deutsch. med. Wchnschr., **60**, 100, 1934.
(13.) **Oppenheimer, A.**: Am. J. Roentgenol., **49**, 49, 1943.
(14.) **Polley, H. F.**: A Study of 1035 Cases of Rheumatoid Spondylitis, Thesis, Graduate School, University of Minnesota, March, 1945. (15.) **Polley, H. F., and Slocumb, C. H.**: Ann. Int. Med., **26**, 240, 1947.
(16.) **Scott, G. S.**: Acta rheumatol., **6**, 7, 1934.

NOTICE

TO THE SUBSCRIBER TO

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Due to the increased costs of material and labor, we find it necessary to increase the subscription price of *The American Journal of the Medical Sciences* to $8.00 per annum beginning January 1, 1948.

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BOOK REVIEWS AND NOTICES

DISEASES OF THE ADRENALS. By LOUIS J. SOFFER, M.D. Pp. 304; 42 ills.; 2 color plates. Phila.: Lea & Febiger, 1946. Price, $5.50.

THIS is a systematic and interestingly written presentation of diseases of the adrenal glands. Physiologic and chemical studies are reviewed in such a way as to give an historical background and to bring up to date the experimental and clinical research which has permitted a scientific approach and understanding of the structural and functional changes seen in man. Certain practical features include the actual techniques of chemical methods used in clinical diagnosis. Case histories and many illustrations have been selected with a view to their usefulness to clinicians. This book can be highly recommended to those interested in theoretical and practical aspects of endocrinology. I. Z.

HOSPITAL CARE IN THE UNITED STATES. A Study of the Function of the General Hospital, Its Rôle in the Care of All Types of Illness, and the Conduct of Activities Related to Patient Service, With Recommendations for Its Extension and Integration for More Adequate Care of the American Public. By the COMMISSION ON HOSPITAL CARE. Pp. 631; 94 tables, 58 charts. New York: The Commonwealth Fund, 1947. Price, $4.50.

THE Commission of 22 members responsible for this report, under the Chairmanship of Thomas S. Gates of the University of Pennsylvania, was drawn from many fields: medicine, public health, hospital administration, nursing, dentistry, social agencies, education, agriculture, industry and labor. The director of the study was A. C. Bachmeyer, M.D.

According to the report, our present rather haphazard development of hospital service will not meet our needs for the future; the base of hospital support must be broadened, and hospital service must be strengthened and made available for all. The Commission completed a pilot study in Michigan. Its procedure, based upon this pilot study, has been adopted in 40 states where hospital surveys are under way.

The report furnishes a plan for analyzing the hospital facilities and needs of any area and a program for integrating and extending hospital service to improve the organization and distribution of hospital care. Quantitative data are abundant and are reflected in the large number of tables and line charts. The statistical analysis of these data is fruitful and stimulating.

The conclusions and recommendations are conservatively formulated. They encourage careful study and analysis of local conditions in each community rather than easy acceptance of superficially conceived general formulas, a commendable feature of this report. The report will interest a wide group of civic minded citizens as well as the members of the professions most directly concerned. J. A.

SIR W. ARBUTHNOT LANE, Bart., C.B., M.S., F.R.C.P., HIS LIFE AND WORK. By W. E. TANNER, M.S., F.R.C.S. Pp. 192; 1 ill. Baltimore: Williams & Wilkins, 1946. Price, $4.50.

THE author of this handsomely written and fair-minded book probably over enthusiastically places Lane "next to Lister on the scroll of the British pioneers of surgery." Like all pioneers and like all great men who "always saw the good in people," Lane was sometimes deceived both by theories and by people, and so was sometimes seriously mistaken. Time has most conspicuously proved him wrong in his views upon intestinal stasis; but, as Mr. Tanner convincingly writes, now in 1946: "The real disorder may have been in the patient's brain or vago-sympathetic system. By his brave and great experiment of colectomy, Lane has proved this. Let us be thankful that no lesser hand has shown this fundamental truth!" This tribute from one surgeon to another is more than loyal and understanding defense; it is sound and leads with admirable logic to the quotation from Carlyle that heads the Epilogue to this

excellent book: "The errors of a wise man are literally more instructive than the truth of a fool." E. T.

CUSHNY'S PHARMACOLOGY AND THERAPEUTICS. By ARTHUR GROLLMAN, Southwestern Medical College, and DONALD SLAUGHTER, Univ. of South Dakota. 13th ed. Pp. 868; 74 ills., Phila.; Lea & Febiger, 1947. Price, $8.50.

IN this edition of a classic text the two new editors have added large sections on recent drugs, making it the first pharmacology textbook to treat streptomycin and other antibiotics, folic acid, antihistamine compounds and the newer sulfonamides. These and other completely new sections are well written, clear, concise and up-to-date. They are responsible in large part for the value of the book as a text for student and physician. It is not only the newer drugs, however, which get such thorough treatment. The 31 page section on digitalis drugs has been revised to include the newer active principles in increasing usage today and a complete consideration of the clinical aspects of digitalis therapy illustrated with numerous electrocardiograms. Turning to other sections, however, one is somewhat disappointed to find the revision spotty and sometimes inadequate. Advances in our understanding of the mechanism of action and therapeutic use of some of the older drugs do not always find their way into the text. More stress on the clinical actions of drugs might be useful to the physician interested in rational therapy and better annotated bibliographies would help the student interested in outside reading. On the other hand the index has been made larger and more complete and the concise listing of official preparations at the end of each section add much to the convenience of the book. S. K.

GYNECOLOGICAL AND OBSTETRICAL PATHOLOGY, WITH CLINICAL AND ENDOCRINE RELATIONS. By EMIL NOVAK, A.B., M.D., D.Sc. (Hon. Dublin), F.A.C.S., Assoc. in Gyn., Johns Hopkins Medical School. Pp. 570; 542 ills.; 15 in color. 2nd ed., Philadelphia and London: W. B. Saunders Co., 1947. Price, $7.50

THIS book was written both for practical value to the clinician and the pathologist. The author is admirably qualified for his task since he is an accomplished clinician who has attained preëminence in the fields of both gynecological endocrinology and gynecological pathology.

The new edition contains 75 more pages and 115 more illustrations than its predecessor. The recent developments in obstetrical and gynecological pathology are included with valuable appraisals of the vaginal smear in the diagnosis of uterine cancer and of the newer concepts concerning erythro-blastosis foetalis.

Two outstanding portions of the book are devoted to carcinoma of the cervix and to ovarian tumors. The microscopic diagnosis, or exclusion of early cervical malignancy, is presented lucidly and the accompanying illustrations are particularly clear. Perhaps it might appear that too much space is devoted to the special ovarian tumors—half of the section—and too little space to the commoner ones, but this apparent overemphasis is justified by recent great interest in the rare ovarian tumors and by the authoritative knowledge of this subject possessed by the writer.

This volume is recommended to both the gynecological pathologist and the clinician because of its great informative value and its easy readability. F. P.

HANDBOOK OF PHYSIOLOGY AND BIOCHEMISTRY. By R. J. S. MCDOWALL, M.D., D.Sc., Professor of Physiology, Univ. of London, King's College. 39th ed. Pp. 898; 305 figs. Phila.: Blakiston, 1946. Price, $7.00.

THERE are not many texts that have stood the test of time covering four years short of a century. Professor McDowall of King's College has been the editor since 1930 when he succeeded Professor Halliburton, who was editor from 1896.

This edition differs from the 38th in that the chapters on histology have been drastically reduced to sections on the relation of function to structure.

This book is still unique in that it brings together information not found in single physiological or biochemical texts of a comparable class.

An interesting commentary on what should be omitted from a medical curriculum is made by the author, namely: "it will do

no harm if the student catches a fleeting glance of things he need not know, for soon he will realize his knowledge of Medicine generally must of necessity be most patchy and superficial." J. S.

A HISTORY OF THE AMERICAN MEDICAL ASSOCIATION, 1847–1947. By MORRIS FISHBEIN, M.D. With the Biographies of the Presidents of the Ass'n. By WALTER L. BIERRING, M.D., and with Histories of the Publications, Councils, Bureaus, and Other Official Bodies. Pp. 1226; 200 ills. Phila., and London: W. B. Saunders, 1947. Price, $10.00.

THIS work, in preparation for several years under the charge of a special committee of the Association, appropriately made its appearance just in time for the Centennial celebration of the Association. Big and weighty as the book is, it is none too large for the accomplishment of its aim—as a record, in the words of the Publication Committee, "of its founder, its founding, its ideals, its motivations and the extent to which these have been accomplished over a period of 100 years." This aim of giving a factual picture as detailed as possible of the largest and probably the most influential medical society that the world has ever seen has been well carried out, so that the book will undoubtedly be permanently the standard source of reference for those seeking information on this subject. It will be a necessary possession of every medical reference library, as well as of most city, county and state medical societies. It is obvious however, that it cannot also serve the individual to any great extent as pleasant continuous reading matter. As this type of reader and even the general public are said to have been in mind, one wonders why objections to a format of two smaller volumes prevailed.

The text contains much, especially that to do with the activities of the last quarter century, that has not previously appeared in print. Following a brief biography of Nathan Smith Davis, who also wrote a history of the Association that he founded, comes Dr. Fishbein's History, occupying almost half the volume. Though the limitations of the factual record mentioned above are apparent here as elsewhere, the well known adroitness and literary ability of the author has kept them at a minimum. Parenthetically, one regrets that "Dr. Pepys" did not choose to lighten his narrative, even in this serious production, with more of his free flowing humor. He has woven into his account interesting considerations of such topics as the early troubles of the Association and its reorganizations, Simmons as editor, quackery, the Journal, animal experimentation, improvement of medical care, the trend toward socialization of medicine, ending with the recent trial by the U. S. Government. When the reader turns to the index to combine such material into connected reading units, its inadequacy—only 16 pages for both persons and subjects—is immediately felt. To the Reviewer this constitutes the most serious defect in the production. Especially for a work written primarily as a factual record, how can the numerous subjects, many turning up many times through the book, be adequately indicated in 8 pages? "Nostrums," for instance, appear in the subject index only as part of the title of a book, though actually receiving lengthy treatment in the libel suit section and several other places. Dr. Bierring's biographical sketches of the 100 presidents, each accompanied by a portrait, occupy 260 pages. Those of which the Reviewer has personal knowledge all appear adequate; together they make a useful reference source. It is of interest, and an indication of the qualifications sought in candidates for this office as well as of the position of organized medicine in this country, that fewer than a third of those elected in the first half century were known to the Reviewer, whereas in the last half century more than half, at the moment at least, have claim to eminence. The remaining space in the book is occupied by brief accounts of the Councils and Bureaus of the Association, and its publications, and various lists gathered into Appendices.

The preparation of the volume was delegated to W. B. Saunders Co., who have brought it out in a form worthy of both Association and publishing house. It is a creditable record of an organization that inevitably has had to struggle with many internal shortcomings; but this perhaps should not even be mentioned when they have been so far outweighed by the many contributions to the advancement and elevation of the practice of medicine of this great Association. E. K.

Rh: Its Relation to Congenital Hemolytic Disease and to Intragroup Transfusion Reactions. By Edith L. Potter, M.D., Ph.D., Ass't. Prof. of Pathology, Dept. of Obstetrics and Gynecology, Univ. of Chicago and Chicago Lying-in Hospital. Pp. 344; 65 ills., 16 tables. Chicago: Year Book Publishers, 1947. Price, $5.50.

This excellent monograph will prove valuable to the clinical laboratory, the blood bank, the obstetrician and pediatrician, and to the general clinician, in a field of great importance, the development of which has occurred almost entirely in the last few years.

In a foreword, L. K. Diamond points out the exceptional qualifications of the author for the task she has undertaken and warmly endorses this work. The history of our knowledge of Rh is reviewed. The characterization of the various Rh antigen and antibodies and the resulting types as they occur in various races is set forth, and the different systems of terminology are compared and related. The description of the Hr factors and of the blocking antibodies is excellent and especially timely The etiology, symptomatology, diagnosis and pathology of "Hemolytic Disease," the name preferred by the author for what has often been called erythroblastosis foetalis, is presented in great detail in a chapter of 160 pages, with excellent illustrations of blood changes and of gross and histological pathology. The significance of the 8 Rh blood types in cases of disputed parentage is tabulated and briefly discussed. Pertinent laboratory techniques are assembled in a final chapter of 36 pages, with photographs and drawings of apparatus and of reactions. The bibliography contains 794 references, extending through at least November, 1946.

While the important contributions of various workers in this field are utilized with due credit given, this work is a unified presentation of the subject based on the experience of the author and not a mere collection of observations from the literature.

Clinician, laboratory worker and student will find the currently available information with respect to this difficult and very important field clearly and readably presented.

J. A.

War Stress and Neurotic Illness. By Abram Kardiner, M. D., Assistant Clinical Professor of Psychiatry, Columbia Univ., with the collaboration of Herbert Spiegel, M.D., formerly Major M.C., A.U.S., Instructor School of Military Neuropsychiatry, Mason General Hospital. Pp. 428; 3 tables. 2nd ed. New York: Paul B. Hoeber, 1947. Price, $4.50.

This study, including 40 case reports, concerns the nervous syndrome consequent upon the stress of war, and variously known as traumatic neurosis, shell shock, battle fatigue, combat exhaustion, and so forth. Though the reason is not definitely known, there were relatively few "extreme hysterical abasias, paralyses, and the epileptiform types of traumatic neurosis in World War II."

Following the Introduction, the chapters are: The Soldier and His Job; Battlefield Psychiatry; The Acute Phase; Treatment of the Acute Phase; The Chronic Phase—Symptomatology; Analysis of the Symptomatology; Development of the Effective Ego; Psychodynamics; Treatment of the Chronic Phase; Course, Prognosis, Differential Diagnosis; Forensic Issues.

Symptoms of the acute phase are those of a cross-section, with the following prodromal manifestations: loss of appetite, carelessness, jumpiness, freezing to the ground, marked irritability, incapacity for relaxation and disturbed sleep; fear or terror sometimes follows. Then the subject may show confusion or depression. Symptoms of the chronic phase vary with the trends shown by the personality. Among the important findings are hypochondriasis, schizophrenia, transference neurosis, defensive ceremonials and tics, autonomic disturbances; psychosomatic disorders, and the epileptic symptom complex. The section on the chronic phase includes the important additions found in the current edition, namely, the uses of barbiturates and hypnosis to render the subject more accessible; the former has the advantage of being an anesthetic, but the disadvantage of blunting the sensorium; the latter, renders the emotional tone more vivid and the reconstruction more effective. This collaborate study is a valuable contribution to our better understanding of the war stress neurosis.

N. Y.

THE TREATMENT OF DIABETES MELLITUS. By ELLIOTT P. JOSLIN, A.M., M.D., Sc.D., Clinical Professor of Medicine Emeritus, Harvard Medical School, Medical Director, Baker Clinic, New England Deaconess Hospital. 8th ed. Pp. 861; 6 ills.; 117 tables. Phila.: Lea & Febiger, 1946. Price, $10.00.

THIS edition of the most complete text on diabetes follows the general outline of the preceding edition. A new chapter on alloxan diabetes affords the best review of this subject to date. Extensive revision of the chapters on the genito-urinary system and on pregnancy in diabetes is noted. Throughout the book the statistics, tables and references have been brought up to 1946, so that the large experience of this Clinic and a wealth of literature is made available in one volume. The 300 or more pages on complications and concurrent conditions are the only such comprehensive source of information on the interrelation of diabetes and other diseases.

One gains the impression that valuable nuggets are often concealed in this mine of information. Improved organization of the book as a whole, more detailed indexing and bibliographies with full titles after each chapter might be helpful. These thoughts for future editions occur to a reviewer who has profound admiration for what is accomplished by this book. F. L.

RADIOLOGY FOR MEDICAL STUDENTS. By F. S. HODGES, M.D., I. LAMPE, M.D., and J. F. HOLT, M.D., Univ. of Michigan. Pp. 424; 103 ills. Chicago: The Year Book Publishers, 1947. Price, $6.75.

THE authors of this book have provided for the medical student a comprehensive, yet brief, insight into Radiology. The book is beautifully prepared on excellent paper with good print and excellent illustration and explanatory drawings. There are 15 chapters, 7 of which are devoted to diagnostic Radiology, and the remainder to therapeutic Radiology.

The modern physician employs Radiology in almost every specialty. It is, therefore, necessary that the medical student become conversant with the indications and limitations of *x*-ray diagnosis. Likewise, it is important for the medical student to be familiar with the various components of the atom and their effect upon tissue. The increasing use of radioactive isotopes in diagnosis and therapy has extended into fields other than Radiology. In fact, they are being used in almost every specialty in medicine. The authors of this book have kept this in mind in preparing their textbook.

It is difficult to avoid being too enthusiastic about this text. It is the type of text that will be exceedingly useful to every teacher in Radiology. E. P.

ADVANCES IN CARBOHYDRATE CHEMISTRY. Vol. II. Edited by W. W. PIGMAN, B.S., A.M., Ph.D., Institute of Paper Chemistry, Appleton, Wis.; M. L. WOLFROM, A.B., M.S., Ph.D., Ohio State Univ., Columbus, Ohio, and STANLEY PEAT, The University, Birmingham, England. Pp. 323. New York: Academic Press, 1946. Price, $6.60.

WITH this 2nd volume "Advances" becomes international in scope. Four chapters are contributed by English chemists and 1 each from France and Canada, while the remaining 4 come from American authors.

The following subjects are reviewed: Melezitose and Turanose, by C. S. Hudson, 34 pages; The Chemistry of Anhydro Sugars, by S. Peat, 39 pages; Analogs of Ascorbic Acid, by F. Smith, 27 pages; Synthesis of Hexitols and Pentitols from Unsaturated Polyhydric Alcohols, by R. Lespieau, 11 pages; The Interrelation of Carbohydrate and Fat Metabolism, by H. J. Deuel, Jr. and M. G. Morehouse, 43 pages; The Chemistry of Nucopolysaccharides and Mucoproteins, by M. Stacey, 39 pages; Bacterial Polysaccharides, by T. H. Evans and H. Hibbert, 29 pages; The Chemistry of Pectic Materials, by E. L. Hirst and J. K. N. Jones, 16 pages; The Polyfructosans and Difructose Anhydrides, by E. J. McDonald, 24 pages; Cellulose Ethers of Industrial Significance, by J. F. Haskins, 14 pages.

All authors are recognized specialists in their topics, and they present a concise summary of present knowledge. The articles by Deuel and Morehouse, Stacey, and Evans and Hibbert in particular are of biochemical interest. Mucopolysaccharides and bacterial polysaccharides have been investigated actively in recent years, yet have not been the subjects of reviews elsewhere. The present reviews are, therefore, appropriate and timely. H. V.

PENICILLIN IN NEUROLOGY. By A. EARL WALKER, M.D., and HERBERT C. JOHNSON, M.D. Pp. 204; 95 ills. Springfield, Ill.: Charles C Thomas, 1946. Price, $5.00.

THIS is an excellent book containing all the latest information, as of 1946, on the use of penicillin in counteracting infection, both acute and chronic, in the central nervous system. Unfortunately, as is the case in all such publications, as new studies and improvements in the therapeutic use of any drug are made, the information rapidly becomes out-of-date.

The treatment of acute or chronic infections of the central nervous system with penicillin faces at once the problem of the passage of the penicillin through the hematoencephalic barrier, so that the drug reaches the cerebrospinal fluid and the substance of the central nervous axis. Even if penicillin does reach the cerebrospinal fluid in rather small amounts, its concentration may not be sufficiently high to affect the more resistant bacteria. For this reason intrathecal injection is necessary for certain diseases. In equivalent antibiotic amounts, calcium penicillin is much more toxic than sodium penicillin.

The first chapters of this monograph outline in considerable detail the difficulties in producing a sufficiently high concentration of penicillin in the cerebrospinal fluid and maintaining it, together with a description of the possible neurologic sequelæ of the intrathecal use of penicillin. One important point, at least, is made clear and emphasized, namely, that intracisternal injection of penicillin is no more effective than lumbar puncture injection and that intraventricular injection of penicillin is no more effective than intracisternal, except in those rather rare cases in which a block between the ventricles and the cistern is present.

Systemically, in the treatment of lues, Herxheimer-like reactions occur with penicillin therapy in about 10% of cases. Furthermore, with the parenteral administration of penicillin, subclinical alterations in brain function may occur during the giving of the drug in somewhat more than 50% of the cases. Following intrathecal administration, meningeal reactions take place with increase in the cell count of the spinal fluid, although the protein content is not altered. Parenchymatous reactions to the lumbar introduction of penicillin in laboratory animals consisted principally in a local radiculitis. Case histories are quoted to show that the same neurologic sequelæ occur in the human following intraspinal administration of penicillin.

Reactions are also noted following cisternal or intraventricular injections of penicillin. An occasional convulsive phenomenon is recorded in the human and in a number of instances, there have been definite changes in the electroencephalographic findings.

The direct application of penicillin to the cortex, or its injection subcortically, in the experimental animal seemed to produce convulsive attacks and electroencephalographic changes from the involved cortical areas. The neuropathologic reactions to the introduction of penicillin into the central nervous system, the authors state, show that the relatively slight reaction of the meninges and brain tissue to the local application of penicillin is in keeping with the few clinical complications following intrathecal penicillin therapy, yet an arachnitis or radiculitis may be produced by the drug. That little, if any, histologic alteration is present in the cerebral cortex, in spite of the severe convulsive effects of the drug, is not surprising, for many convulsive agents exert their effect on the brain without constant demonstrable microscopic findings.

As the first 5 chapters of this monograph appear to aim at emphasizing the bad effects of penicillin, one wonders whether the use of penicillin is justifiable. However, the second 5 chapters are devoted to the clinical results, and the potential disasters following the use of penicillin in the treatment of infections of the central nervous system are, for the most part, ignored.

The literature is carefully reviewed and very exact and detailed descriptions given of the methods for using penicillin, with or without sulfadiazine, in the treatment of meningitis, brain abscess, and other acute and chronic infections of the central nervous system. This is, to the Reviewer at least, the more interesting and valuable part of the book. The text is clear and concise; the clinical examples, well chosen and presented; so that these chapters contain a mass of information which has been skillfully made available to the profession at large. The Reviewer strongly advises every neurosurgeon to read carefully Chapters 6 to 10 in this monograph.

A final chapter is devoted to the results with streptomycin and the other newer antibiotics. With the exception of streptomycin, the other materials are so recently developed that but little information, other than from the laboratory, is available.

This is an excellent monograph, carefully prepared and well annotated, as one would expect from its senior author. The laboratory data are important in showing that, while penicillin is an extremely effective drug against certain organisms in sufficient concentration, nevertheless, its use in large amounts in the central nervous system is not without its dangers. The proper technique to be used is given in detail so that any of the unfortunate side effects, which seem to be inherent in the use of penicillin, can, for the most part, be avoided. The book is heartily recommended to all those who deal with infection in the central nervous system. F.G.

NEW BOOKS

Physikalische Medizin in Diagnostik und Therapie. By WOLFGANG HOLZER, PROF. DR. MED. ET DR. ING., Vorstand der Psychiatrisch - Neurologischen Universitätsklinik Graz. (Bücherei der Physikalischen Medizin, Band II.) Pp. 769; 379 ills. Vienna, Austria: Wilhelm Maudrich; New York: Grune & Stratton, 1947. Price, $9.00.

Neuropathology, Its Clinicopathologic Aspects. By I. MARK SCHEINKER, M.D. Foreword by TRACY J. PUTNAM, M.D. Pp. 306; 208 ills. Springfield, Ill.: Charles C Thomas, 1947. Price, $6.75.

Endoskopie der Harnröhre. By DR. ALOIS GLINGAR, Facharzt fur Urologie. (Weiner Beiträge zur Urologie, Band II.) Pp. 68; 36 ills. Vienna, Austria: Wilhelm Maudrich; New York: Grune & Stratton, 1947. Price, $4.00.

Erkrankungen des Uropoetischen Systems und der Prostata Durch Störung der Blutströmung. By PROF. DR. KARL HUTTER. (Wiener Beuträge zur Urologie, Band I.) Pp. 200; 40 ills. Vienna, Austria: Wilhelm Maudrich; New York: Grune & Stratton, 1947. Price, $4.00.

Food Regulations and Compliance. By ARTHUR D. HERRICK. Vol. 2. Pp. 655. New York: Revere Publishing Co., 1947. Price, $10.00.

Hospital Care in the United States. By THE COMMISSION ON HOSPITAL CARE. Pp. 631; 94 tables; 58 charts. New York: Commonwealth Fund, 1947. Price, $4.50. Reviewed in this issue.

Communal Sick-Care in the German Ghetto. By JACOB R. MARCUS, PH.D., Adolph S. Ochs Prof. of Jewish History. Pp. 335. Cincinnati: The Hebrew Union College Press, 1947. Price, $2.50.

Office Treatment of the Eye. By ELIAS SELINGER, M.D., Attending Ophthalmologist, Mount Sinai, Cook County and Michael Reese Hosps. Pp. 542; 67 ills. Chicago: Year Book Publishers, 1947. Price, $7.75.

The Medical Writings of Anonymus Londinensis. By W. H. S. JONES, LITT.D., F.B.A. Pp. 168. Cambridge: The University Press; New York: The Macmillan Company, 1947. Price, $2.75.

Dying, Apparent Death and Resuscitation. By S. JELLINEK, M.D., Prof. of Electropathology, Univ. of Vienna, Laureat de l'Institut de France. Pp. 263. Baltimore: Williams & Wilkins, 1947. Price, $5.50.

Pharmaco-therapeutic Notebook. By H. W. TOMSKI, Diploma in Biochemical Analysis. Foreword by HUGH A. DUNLOP, M.D., M.Sc., F.R.C.P. Pp. 280. Baltimore and London: Williams & Wilkins, 1947. Price, $4.50.

The Rotunda Hospital. By O'DONNEL T. D. BROWN, M.B., M.A., M.A.O. (UNIV. DUB.), F.R.C.P. (I.), F.R.C.O.G. Pp. 296; 44 ills. Baltimore and London: Williams & Wilkins, 1947. Price, $11.00.

Nutritional Disorders of the Nervous System. By JOHN D. SPILLANE, B.Sc., M.D. WALES), M.R.C.P. (LOND.). Foreword by GEORGE RIDDOCH, M.D., F.R.C.P. Pp. 280; 103 ills. Baltimore and London: Williams & Wilkins, 1947. Price, $5.00.

A Study of Individual Children's Diets. By E. M. WIDDOWSON, B.Sc., PH.D., Medical Research Council. Special Report Series No. 257. Pp. 196. London: His Majesty's Stationery Office, 1947. Price, 6s.

A STUDY of 1028 British children whose individual food consumption has been recorded, giving data under some 20 headings, such as calories, proteins, vitamins and so on—and on the diets of twins, diabetics, children of unemployed and their results. "The one outstanding fact, which has been brought out again and again by this investigation, is that similar individuals may differ enormously and unpredictably in their food habits."

NEW EDITIONS

Symptoms and Signs in Clinical Medicine. By E. NOBLE CHAMBERLAIN, M.D., M.Sc., F.R.C.P., Lecturer in Medicine, Univ. of Liverpool. 4th ed. Pp. 463; 346 ills., 9 in color. Baltimore and London: Williams & Wilkins, 1947. Price, $8.00.

Massage and Remedial Exercises in Medical and Surgical Conditions. By NOËL M. TIDY, Sister-in-Charge, Red Cross Massage Clinic, High Wycombe. 7th ed. Pp. 480; 190 ills. Baltimore and London: Williams & Wilkins, 1947. Price, $6.00.

Developmental Diagnosis, Normal and Abnormal Child Development. By ARNOLD GESELL, M.D., and CATHARINE S. AMATRUDA, M.D. 2nd ed. Pp. 496; 22 figs. New York: Paul B. Hoeber, 1947. Price, $7.50.

The American Illustrated Medical Dictionary. By W. A. NEWMAN DORLAND, A.M., M.D., F.A.C.S. 21st ed. Pp. 1660; 880 ills., including 233 portraits. Philadelphia and London, W. B. Saunders, 1947. Price, $8.00 without Thumb Index; $8.50 with Thumb Index.

Internal Medicine in General Practice. By ROBERT PRATT MCCOMBS, B.S., M.D., F.A.C.P. 2nd ed. Pp. 741; 122 ills. Philadelphia and London: W. B. Saunders, 1947. Price, $8.00.

A Textbook of Pathology. By WILLIAM BOYD, M.D., Prof. of Pathology and Bacteriology of the Univ. of Toronto. 5th ed. Pp. 1049; 500 ills.; 30 color plates. Philadelphia: Lea & Febiger, 1947. Price, $10.00.

IN the 4 years that have elapsed since the last revision, much new and important material has become available; accordingly the author has made many additions and revisions that add considerably to the textbook's value, *e. g.*, sections on the kidney in anoxia and in sulfonamide poisoning; the lung in Löffler's pneumonia. Material has been added on cardiac infarction, hypertension heart disease, the structure of the coronary arteries, and on many other topics. (M. McC.)

An Introduction to Biochemistry. By WILLIAM ROBERT FEARON, M.A., Sc.D., M.B., Prof. of Biochemistry, Univ. of Dublin. 3rd ed. Pp. 569. New York: Grune & Stratton, 1947. No price given.

THIS revised edition of a brief, elementary text, which has been quite popular in England, is well organized and readable. The subject matter appears to recommend it largely for general undergraduate courses in biochemistry. While there are practical medical applications included, phases of the subject such as gas transport, acid-base, water balance, and metabolism are not treated with the degree of detail this Reviewer would like to see in a modern text for medical students. The Bibliographies at the end of each chapter refer the reader practically solely to other texts and review articles. This device saves space and effort, but it leads to a dogmatic and compendium type of presentation. (D. D.)

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